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(71) Applicant (for all designated States except US): **ANTI-SOMA RESEARCH LIMITED** [GB/GB]; West Africa House, Hanger Lane, Ealing, London W5 3QR (GB).

(72) Inventor; and

(75) Inventor/Applicant (for US only): **YOUNG, Robert, James** [GB/GB]; Antisoma Research Limited, West Africa House, Hanger Lane, Ealing, London W5 3QR (GB).

(74) Agent: **THOMAS, Philip, J., D.**; Eric Potter Clarkson, Park View House, 58 The Ropewalk, Nottingham NG1 5DD (GB).

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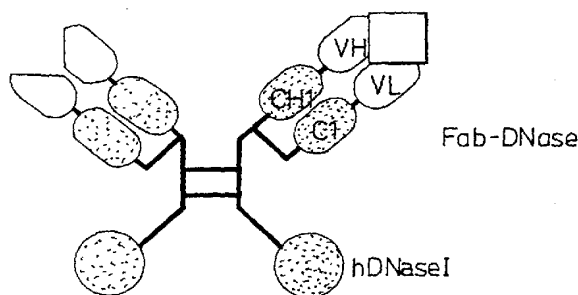
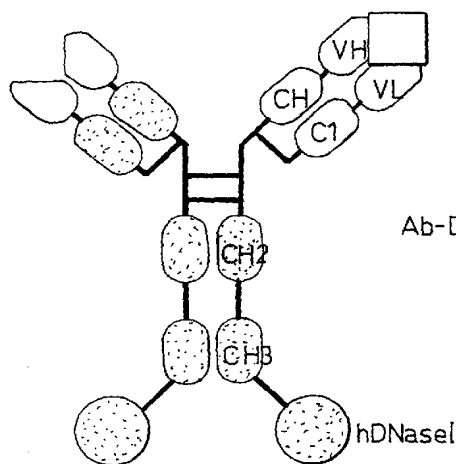
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(54) Title: COMPOUNDS FOR TARGETING



(57) **Abstract:** A compound comprising a target cell-specific portion and a cytotoxic portion characterised in that the target cell-specific portion comprises a humanised monoclonal antibody having specificity for polymorphic epithelial mucin (PEM), or an antigen binding fragment thereof, and the cytotoxic portion has endonucleolytic activity. Preferably, the target cell-specific portion comprises a humanised HMFG-1 antibody or an antigen binding fragment thereof. Advantageously, the cytotoxic portion is at least the catalytically active portion of a DNA endonuclease, e.g. a human DNA endonuclease I. The invention further provides nucleic acids encoding the compounds of the invention, and the use of such compounds in medicine, e.g. in the treatment of cancer.



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**CLAIMS**

1. A compound comprising a target cell-specific portion and a cytotoxic portion characterised in that:  
  
(i) the target cell-specific portion comprises an humanised monoclonal antibody having specificity for polymorphic epithelial mucin (PEM), or an antigen binding fragment thereof; and  
  
(ii) the cytotoxic portion has endonucleolytic activity.
2. A compound according to Claim 1 wherein the target cell-specific portion comprises an humanised HMFG-1 antibody or an antigen binding fragment thereof.
3. A compound according to Claim 2 wherein the target cell-specific portion is an humanised HMFG-1 antibody.
4. A compound according to Claim 1 or 2 wherein the target cell-specific portion comprises an antigen binding fragment of the humanised antibody selected from the group consisting of Fab-like molecules, such as Fab and F(ab')<sub>2</sub>, Fv molecules, disulphide-linked Fv molecules, ScFv molecules and single domain antibodies (dAbs).
5. A compound according to Claim 4 wherein the target cell-specific portion comprises a Fab molecule.
6. A compound according to Claim 4 wherein the target cell-specific

portion comprises a  $F(ab')_2$  molecule.

7. A compound according to Claim 1 wherein the target cell-specific portion comprises an amino acid sequence encoded by at least part of one or both of the nucleotide sequences of Figure 3(a) and (d).
8. A compound according to Claim 7 wherein the target cell-specific portion comprises an amino acid sequence encoded by the nucleotide sequence of Figure 3(a) and an amino acid sequence encoded by the nucleotide sequence of Figure 3(d).
9. A compound according to any one of Claims 1 to 8 wherein the cytotoxic portion has DNA endonucleolytic activity.
10. A compound according to Claim 9 wherein the cytotoxic portion is at least the catalytically active portion of a DNA endonuclease.
11. A compound according to Claim 10 wherein the endonuclease is a mammalian deoxyribonuclease I.
12. A compound according to Claim 11 wherein the endonuclease is a human deoxyribonuclease I.
13. A compound according to Claim 1 wherein the endonuclease is a restriction endonuclease.
14. A compound according to Claim 10 wherein the cytotoxic portion comprises the amino acid sequence shown in Figure 2(a) or (b).

15. A compound according to any one of Claims 1 to 14 wherein a nuclear localization signal is incorporated.
16. A compound according to Claim 15 wherein the nuclear localization signal comprises the sequence PKKKRKV.
17. A compound according to any one of Claims 1 to 16 wherein the target cell-specific portion and the cytotoxic portion are fused.
18. A compound according to Claim 17 wherein the target cell-specific portion and the cytotoxic portion are separated by a linker sequence.
19. A compound according to Claim 18 wherein the linker sequence is or comprises GG or GSGG.
20. A compound according to any one of Claims 1 to 19 wherein the compound comprises all or part of the amino acid sequence as shown in Figure 3(c) together with all or part of an amino acid sequence selected from the group consisting of amino acid sequences as shown in Figures 5(d), 6(d), 7(b), 8(b), 9(b), 10(b), 11(b), 12(b), 13(d), 14(d), 15(d), 16(c), 17(d), 18(d) and 19(d).
21. A compound according to Claim 20 wherein the compound comprises an amino acid sequence as shown in Figure 3(c) and an amino acid sequence as shown in Figure 7(b).
22. A compound according to Claim 20 wherein the compound comprises

an amino acid sequence as shown in Figure 3(c) and an amino acid sequence as shown in Figure 14(d).

23. A nucleic acid molecule encoding a compound as defined in any one of Claims 1 to 22.
24. A nucleic acid molecule according to Claim 23 wherein the molecule comprises all or part of the nucleotide sequence as shown in Figure 3(a or b) together with all or part of a nucleotide sequence selected from the group consisting of nucleotide sequences as shown in Figures 5(a, b and c), 6(a, b and c), 7(a), 8(a), 9(a), 10(a), 11(a), 12(a), 13(a, b and c), 14(a, b and c), 15(a, b and c), 16(a and b), 17(a, b and c), 18(a, b and c) and 19(a, b and c).
25. A nucleic acid molecule according to Claim 24 wherein the molecule comprises a nucleotide sequence as shown in Figure 3(b) and a nucleotide sequence as shown in Figure 7(a).
25. A nucleic acid molecule according to Claim 24 wherein the molecule comprises a nucleotide sequence as shown in Figure 3(b) and a nucleotide sequence as shown in Figure 14(c).
26. A nucleic acid molecule according to any one of Claims 23 to 25 wherein the molecule further comprises a Kozak consensus ribosome-binding site.
27. A vector comprising a nucleic acid molecule according to any one of Claims 23 to 26.

28. A host cell comprising a vector according to Claim 27.
29. A pharmaceutical composition comprising a compound according to any one of Claims 1 to 22 and a pharmaceutically acceptable carrier.
30. A compound according to any one of Claims 1 to 22 for use in medicine.
31. Use of a compound according to any one of Claims 1 to 22 in the preparation of a medicament for treating a mammal having said target cells to be destroyed.
32. A method of treating a mammal having target cells to be destroyed, the method comprising administering a compound according to any one of Claims 1 to 22 to said mammal.
33. A use according to Claim 31 or a method according to Claim 32 wherein the mammal is a human.
34. A use according to Claim 31 or a method according to Claim 32 wherein the target cells to be destroyed are cancer cells.
35. A use or a method according to Claim 34 wherein the cancer cells are epithelial cancer cells.
36. A use or a method according to Claim 35 wherein the cancer cells are ovarian, gastric, colorectal and/or pancreatic cancer cells.

37. A use or a method according to Claim 36 wherein the cancer cells are ovarian cancer cells.
38. A compound substantially as described herein, preferably with reference to one or more of the accompanying figures.

## COMPOUNDS FOR TARGETING

The present invention relates to cytotoxic compounds that have a high  
5 avidity for, and can be targeted to, selected cells. Specifically, the  
invention provides compounds comprising a cytotoxic portion having  
DNA endonucleolytic activity and a target-cell specific portion having  
specificity for human polymorphic epithelial mucin (PEM).

### 10 **Background**

The cell-specific targeting of compounds that are directly, or indirectly,  
cytotoxic has been proposed as a way to combat diseases such as cancer.  
Bagshawe and his co-workers have disclosed (Bagshawe (1987) *Br. J.*  
15 *Cancer* **56**, 531; Bagshawe *et al* (1988) *Br. J. Cancer* **58**, 700; WO  
88/07378) conjugated compounds comprising an antibody or part thereof  
and an enzyme, the antibody being specific to tumour cell antigens and the  
enzyme acting to convert an innocuous pro-drug into a cytotoxic  
compound. The cytotoxic compounds were alkylating agents, *e.g.* a  
20 benzoic acid mustard released from *para*-N-bis(2-  
chloroethyl)aminobenzoyl glutamic acid by the action of *Pseudomonas sp.*  
CPG2 enzyme.

An alternative system using different pro-drugs has been disclosed  
25 (WO 91/11201) by Epenetos and co-workers. The cytotoxic compounds  
were cyanogenic monosaccharides or disaccharides, such as the plant  
compound amygdalin, which release cyanide upon the action of a  $\beta$ -  
glucosidase and hydroxynitrile lyase.

In a further alternative system, the use of antibody-enzyme conjugates containing the enzyme alkaline phosphatase in conjunction with the pro-drug etoposide 4'-phosphate or 7-(2'-aminoethyl phosphate)mitomycin or  
5 a combination thereof have been disclosed (EP 0 302 473; Senter *et al* (1988) *Proc. Natl. Acad. Sci. USA* **85**, 4842).

Rybak and co-workers have disclosed (Rybak *et al* (1991) *J. Biol. Chem.* **266**, 21202; WO 91/16069) the cytotoxic potential of a monomeric  
10 pancreatic ribonuclease when injected directly into *Xenopus* oocytes and the cytotoxic potential of monomeric RNase coupled to human transferrin or antibodies directed against the transferrin receptor. The monomeric RNase hybrid proteins were cytotoxic to human erythroleukaemia cells *in vitro*.

15 Other approaches are the *in vivo* application of streptavidin conjugated antibodies followed, after an appropriate period, by radioactive biotin (Hnatowich *et al* (1988) *J. Nucl. Med.* **29**, 1428-1434), or injection of a biotinylated mAb followed by radioactive streptavidin (Paganelli *et al*  
20 (1990) *Int. J. Cancer* **45**, 1184-1189). A pilot radioimmunolocalisation study in non-small cell lung carcinomas was conducted with encouraging results (Kalofonos *et al* (1990) *J. Nucl. Med.* **31**, 1791-1796).

Apart from these examples, it is rather more common to see biotinylated  
25 antibodies and streptavidin-enzyme conjugates, which are used in enzyme-linked immunosorbent assays.

These previous systems have used relatively large antibody-enzyme,

antibody-streptavidin or antibody-biotin conjugates and may comprise portions of non-mammalian origin which are highly immunoreactive.

We have now devised improved compounds for targeting cells to be  
5 destroyed.

### Summary of Invention

A first aspect of the invention provides a compound comprising a target  
10 cell-specific portion and a cytotoxic portion characterised in that the target cell-specific portion comprises an humanised monoclonal antibody having specificity for polymorphic epithelial mucin (PEM), or an antigen binding fragment thereof, and the cytotoxic portion has endonucleolytic activity.

15 By "target cell specific" portion we mean the portion of the compound which comprises one or more binding sites which recognise and bind to polymorphic epithelial mucin (PEM) on the target cell. Upon contact with the target cell, the target cell specific portion is preferably internalised along with the cytotoxic portion. Such internalisation results in the  
20 cytotoxic portion being delivered to the cell cytosol, where it has access to the cell's nucleic acid molecules.

The target cell-specific portion of the compounds of the invention comprises an humanised monoclonal antibody having specificity for  
25 polymorphic epithelial mucin (PEM), or an antigen binding fragment thereof.

Polymorphic epithelial mucin, or PEM, is a component of the human milk

fat globule. PEM is expressed by cells in several body tissues and is also found in urine. Significantly, PEM is known to be expressed in epithelial cancer cells, notably in ovarian, gastric, colorectal and pancreatic cancer cells.

5

Monoclonal antibodies which will bind to PEM are already known, but in any case, with today's techniques in relation to monoclonal antibody technology, antibodies can be prepared to most antigens. The antigen-specific portion may be a whole antibody, a part of an antibody (for example a Fab or F(ab')<sub>2</sub> fragment), a synthetic antibody fragment (for example a single chain Fv fragment [ScFv]), or a peptide/peptidomimetic or similar. Suitable monoclonal antibodies to selected antigens may be prepared by known techniques, for example those disclosed in *"Monoclonal Antibodies: A manual of techniques"*, H Zola (CRC Press, 1988) and in *"Monoclonal Hybridoma Antibodies: Techniques and Applications"*, J G R Hurrell (CRC Press, 1982) and *Antibody Engineering, A Practical Approach*, McCafferty, J. et al, ed. (IRL Pres, 1996).

20 By 'humanised monoclonal antibody' we include monoclonal antibodies having at least one chain wherein the framework regions are predominantly derived from a first, acceptor monoclonal antibody of human origin and at least one complementarity-determining region (CDR) is derived from a second, donor monoclonal antibody having specificity  
25 for PEM. The donor monoclonal antibody may be of human or non-human origin, for example it may be a murine monoclonal antibody.

Preferably, both chains of the humanised monoclonal antibody comprise

CDRs grafted from a donor monoclonal antibody having specificity for PEM.

Advantageously, the CDR-grafted (*i.e.* humanised) chain comprises two  
5 or all three CDRs derived from a donor antibody having specificity for PEM.

Conveniently, the humanised monoclonal antibody comprises only human  
framework residues and CDRs from a donor antibody having specificity  
10 for PEM.

However, it will be appreciated by those skilled in the art that in order to  
maintain and optimise the specificity of the humanised antibody it may be  
necessary to alter one or more residues in the framework regions such that  
15 they correspond to equivalent residues in the donor antibody.

Conveniently, the framework regions of the humanised antibody are  
derived from an human IgG monoclonal antibody.

20 Methods of making humanised monoclonal antibodies are well-known in  
the art, for example see Jones *et al.* (1986) *Nature* **321**:522-525,  
Riechmann *et al.* (1988) *Nature* **332**:323-327, Verhoeyen *et al.* (1988)  
*Science* **239**:1534-1536 and EP 239 400 (to Winter).

25 In a preferred embodiment of the first aspect of the invention, the target  
cell-specific portion comprises an humanised HMFG-1 monoclonal  
antibody or an antigen binding fragment thereof.

HMFG antibodies are raised against human milk fat globule (HMFG), in a delipidated state (see Taylor-Papadimiriou *et al.*, 1981, *Int. J. Cancer* 28:17-21 and Gendler *et al.*, 1988, *J. Biol. Chem.* 236:1282-12823). HMFG-1 monoclonal antibodies bind to a particular component of  
5 HMFG, namely polymorphic epithelial mucin (PEM). Binding is thought to involve the amino acid sequence APDTR within the twenty amino acid tandem repeats of the *muc-1* gene product.

Exemplary humanised HMFG-1 antibodies are disclosed in WO 92/04380.  
10 Advantageously, the target cell-specific portion is an humanised HMFG-1 monoclonal antibody.

In a preferred embodiment of the first aspect of the invention, the target  
15 cell-specific portion comprises a fragment of an humanised monoclonal antibody having specificity for polymorphic epithelial mucin (PEM), said fragment retaining the antigen binding properties of the parent antibody.

The variable heavy ( $V_H$ ) and variable light ( $V_L$ ) domains of the antibody  
20 are involved in antigen recognition, a fact first recognised by early protease digestion experiments. Further confirmation was found by "humanisation" of rodent antibodies. Variable domains of rodent origin may be fused to constant domains of human origin such that the resultant antibody retains the antigenic specificity of the rodent parented antibody  
25 (Morrison *et al* (1984) *Proc. Natl. Acad. Sci. USA* 81, 6851-6855).

That antigenic specificity is conferred by variable domains and is independent of the constant domains is known from experiments involving

the bacterial expression of antibody fragments, all containing one or more variable domains. These molecules include Fab-like molecules (Better *et al* (1988) *Science* **240**, 1041); Fv molecules (Skerra *et al* (1988) *Science* **240**, 1038); disulphide-linked Fv molecules (Young *et al.*, 1995, *FEBS Lett.* **377**:135-139); single-chain Fv (ScFv) molecules where the V<sub>H</sub> and V<sub>L</sub> partner domains are linked via a flexible oligopeptide (Bird *et al* (1988) *Science* **242**, 423; Huston *et al* (1988) *Proc. Natl. Acad. Sci. USA* **85**, 5879) and single domain antibodies (dAbs) comprising isolated V domains (Ward *et al* (1989) *Nature* **341**, 544). A general review of the techniques involved in the synthesis of antibody fragments which retain their specific binding sites is to be found in Winter & Milstein (1991) *Nature* **349**, 293-299.

By "ScFv molecules" we mean molecules wherein the V<sub>H</sub> and V<sub>L</sub> partner domains are linked via a flexible oligopeptide.

Chimaeric antibodies are discussed by Neuberger *et al* (1988, *8th International Biotechnology Symposium Part 2*, 792-799).

The advantages of using antibody fragments, rather than whole antibodies, are several-fold. The smaller size of the fragments allows for rapid clearance, and may lead to improved tumour to non-tumour ratios. Fab, Fv, ScFv, disulphide Fv and dAb antibody fragments can all be expressed in and secreted from bacteria, such as *E. coli*, or eukaryotic expression systems such as Yeast or mammalian systems, thus allowing the facile production of large amounts of the said fragments.

Whole antibodies, and F(ab')<sub>2</sub> fragments are "bivalent". By "bivalent" we

mean that the said antibodies and  $F(ab')_2$  fragments have two antigen combining sites. In contrast, Fab, Fv, ScFv, disulphide Fv and dAb fragments are monovalent, having only one antigen combining site.

- 5 Preferably, the target cell-specific portion of the compounds of the invention comprises an antigen binding fragment of the humanised antibody selected from the group consisting of Fab-like molecules, such as Fab and  $F(ab')_2$ , Fv molecules, disulphide-linked Fv molecules, ScFv molecules and single domain antibodies (dAbs).

10

More preferably, the target cell-specific portion comprises a Fab molecule or a  $F(ab')_2$  molecule.

- Yet more preferably, the target cell-specific portion comprises an amino acid sequence encoded by at least part of one or both of the nucleotide sequences of Figure 3(a) and (d).
- 15

- Most preferably, the target cell-specific portion comprises an amino acid sequence encoded by the nucleotide sequence of Figure 3(a) and an amino acid sequence encoded by the nucleotide sequence of Figure 3(d).
- 20

Preferably, the target cell-specific portion recognises the target cell with high avidity.

- 25 By "high avidity" we mean that the target cell-specific portion recognises the target cell with a binding constant of at least  $K_d = 10^{-6}$  M, preferably at least  $K_d = 10^{-9}$  M, suitably  $K_d = 10^{-10}$  M, more suitably  $K_d = 10^{-11}$  M, yet more suitably still  $K_d = 10^{-12}$  M, and more preferably  $K_d = 10^{-15}$  M or

even  $K_d = 10^{-18}$  M.

Preferably, the target cell-specific portion comprises an antigen binding fragment of an humanised HMFG-1 monoclonal antibody, *e.g.* an Fab or  
5 F(ab')<sub>2</sub> fragment thereof, wherein a hinge region contains a mutation (*i.e.* wherein the hinge is a variant or hybrid of a naturally occurring hinge). More preferably, the variant hinge comprises the amino acid sequence CCVECPPCPAPE.

10 By 'cytotoxic portion' we mean a portion having endonucleolytic activity which is toxic to the cell if it is to reach, and preferably enter said cell.

In a preferred embodiment of the first aspect of the invention, the cytotoxic portion has DNA endonucleolytic activity.

15

Advantageously, the cytotoxic portion is at least the catalytically active portion of a DNA endonuclease.

Examples of known DNA endonucleases include bovine DNase I (see  
20 Worrall and Conolly, 1990, *J. Biol. Chem.* **265**:21889-21895). Human pancreatic DNase I has also been cloned (see Shak *et al.*, 1990, *Proc. Natl. Acad. Sci. USA* **87**:9188-9192 and Hubbard *et al.*, 1992, *New Eng. J. Med.* **326**:812-815).

25 Preferably, the endonuclease is a mammalian deoxyribonuclease I.

More preferably, the endonuclease is a human deoxyribonuclease I.

Most preferably, the cytotoxic portion comprises the amino acid sequence shown in Figure 2(a) or 2(b).

Preferably, the cytotoxic portion of the compound of the invention is  
5 capable of oligomerisation, *e.g.* dimerisation. Attachment of the target-cell specific portion to a cytotoxic portion capable of oligomerisation provides a method for increasing the number of binding sites to the target cell. For example, if the target cell-specific portion is joined to a portion capable of forming a dimer then the number of target cell-specific binding  
10 sites is two; if the target cell-specific portion is joined to a portion capable of forming a tetramer then the number of target cell-specific binding sites is four. The number of target cell-specific binding sites is greater than one and the compounds may therefore have a greater avidity for the target cell than do compounds which only have one target cell-specific binding  
15 site.

It is preferable for the cytotoxic portion of the compound of the invention capable of oligomerisation to contain no interchain disulphide bonds nor intrachain disulphide bonds; to be well characterised; to be non-toxic; to  
20 be stable; to be amenable to preparation in a form suitable for pre-clinical or clinical use or be in pre-clinical or clinical use; and for the subunit monomers to have a high affinity for each other, that is they contain one or more subunit binding sites.

25 Advantageously, the cytotoxic portion is of mammalian, preferably human, origin. The use of the said mammalian proteins as the cytotoxic portion of the compound of the invention is advantageous since such compounds are less likely to give rise to undesirable immune reactions.

It will be appreciated by those skilled in the art that the cytotoxic portion may be a variant of a naturally occurring endonuclease.

- 5 By “a variant” we include cytotoxic portions comprising of a naturally occurring endonuclease wherein there have been amino acid insertions, deletions or substitutions, either conservative or non-conservative, such that the changes do not substantially reduce the endonuclease activity of the variant compared to that of the naturally occurring endonuclease. For  
10 example, the variant may have increased activity compared to the naturally occurring endonuclease

Such variants may be made using methods of protein engineering and site-directed mutagenesis commonly known in the art (for example, see  
15 Sambrook *et al.*, 1989, *Molecular cloning: A Laboratory Manual*, 2<sup>nd</sup> edition, Cold Spring Harbor Laboratory Press, NY, USA).

In an alternative embodiment, the endonuclease is a restriction endonuclease, such as a microbial type II restriction endonuclease.  
20 Exemplary type II restriction endonucleases include *Bam*HI, *Hind*III, *Msp*I, *Sau*3AI, *Hin*fI, *Not*I and *Eco*RI.

In another preferred embodiment of the first aspect of the invention, a nuclear localization signal is incorporated into the compound.

25

Preferably, the nuclear localization signal (NLS) comprises a nuclear localization signal from the SV40 large T antigen (Kalderon *et al.*, 1984, *Cell* 39:499-509), and specifically the amino acid sequence PKKKRKV.

Inclusion of a nuclear localization signal encourages the compound of the invention to gain access to the chromosomal DNA during the periods of the cell cycle when the nuclear membrane is intact, since the nuclear pores are permeable to large molecules incorporating said nuclear localization  
5 signal.

In a further preferred embodiment of the first aspect of the invention, the target cell-specific portion and the cytotoxic portion are fused to create a fusion compound.

10

By "fusion compound" we include a compound comprising one or more functionally distinct portions, wherein the distinct portions are contained within a single polypeptide chain produced by recombinant DNA techniques. For example, the compound may comprise a whole antibody  
15 wherein the heavy chain is fused to human DNase I. Alternatively, the compound may comprise an Fab or F(ab')<sub>2</sub> fragment of an antibody wherein the truncated heavy chain (*i.e.* the Fd chain) is fused to human DNase I.

20 Preferably, the target-cell specific and the cytotoxic portion of the fusion compound of the invention separated by a linker sequence, for example to allow greater flexibility of the portions relative to one another.

More preferably, the linker sequence comprises a GG dipeptide.

25

Most preferably the linker sequence is or comprises GG or GSGG.

Alternatively, the target-cell specific and the cytotoxic portion of the

compound of the invention are separate moieties linked together by any of the conventional ways of cross-linking polypeptides, such as those generally described in O'Sullivan *et al Anal. Biochem.* (1979) **100**, 100-108. For example, the antibody portion may be enriched with thiol  
5 groups and the enzyme portion reacted with a bifunctional agent capable of reacting with those thiol groups, for example the N-hydroxysuccinimide ester of iodoacetic acid (NHIA) or N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP). Amide and thioether bonds, for example achieved with m-maleimidobenzoyl-N-hydroxysuccinimide ester,  
10 are generally more stable *in vivo* than disulphide bonds.

In a preferred embodiment of the first aspect of the invention, the compound comprises all or part of the amino acid sequence as shown in Figure 3(c) (*i.e.* an HMFG-1 light chain) together with all or part of an  
15 amino acid sequence selected from the group consisting of amino acid sequences as shown in Figures 5(d), 6(d), 7(b), 8(b), 9(b), 10(b), 11(b), 12(b), 13(d), 14(d), 15(d), 16(c), 17(d), 18(d) and 19(d) (*i.e.* an HMFG-1 heavy or Fd chain/DNase fusion).

20 Advantageously, the compound is a whole HMFG-1 antibody/human DNase I fusion compound comprising an amino acid sequence as shown in Figure 3(c) and an amino acid sequence as shown in Figure 7(b). Preferably, the compound is a tetrameric compound comprising two HMFG-1 light chains and two HMFG-1 heavy chain /DNase I fusions.

25

Conveniently, the compound comprises an amino acid sequence as shown in Figure 3(c) and an amino acid sequence as shown in Figure 14(d).

Preferably, the compound comprises one of the pairs of amino acid sequences defined above wherein the leader sequence of each amino acid (the first 19 amino acids of the sequences shown in each figure) is removed. It will be appreciated by persons skilled in the art that the compounds of the invention may also comprise variants of such amino acid sequences.

Suitably, the compound is a tetrameric compound comprising two HMFG-1 light chains and two HMFG-1 Fd chain /DNase I fusions. More preferably, the compound is a dimeric compound comprising one HMFG-1 light chain and one HMFG-1 Fd chain /DNase I fusion.

A second aspect of the invention provides a nucleic acid molecule encoding a compound according to the first aspect of the invention, or a target cell-specific portion or cytotoxic portion thereof.

By "nucleic acid molecule" we include DNA, cDNA and mRNA molecules.

In a preferred embodiment of the second aspect of the invention, the nucleic acid molecule comprises all or part of the nucleotide sequence as shown in Figure 3(a or b) (*i.e.* encoding an HMFG-1 light chain) together with all or part of a nucleotide sequence selected from the group consisting of nucleotide sequences as shown in Figures 5(a, b and c), 6(a, b and c), 7(a), 8(a), 9(a), 10(a), 11(a), 12(a), 13(a, b and c), 14(a, b and c), 15(a, b and c), 16(a and b), 17(a, b and c), 18(a, b and c) and 19(a, b and c) (*i.e.* encoding an HMFG-1 heavy or Fd chain/DNase fusion).

Advantageously, the nucleic acid molecule comprising a nucleotide sequence as shown in Figure 3(b) and a nucleotide sequence as shown in Figure 7(a).

- 5 Conveniently, the compound comprises a nucleotide sequence as shown in Figure 3(b) and a nucleotide sequence as shown in Figure 14(c).

Alternatively, the nucleic acid molecule comprises nucleotide sequences that are degenerate sequences of those nucleotide sequences identified  
10 above (*i.e.* which encode the same amino acid sequence).

A further aspect of the present invention provides a method of making a compound according to the first aspect of the invention, said method comprising expressing one or more nucleic acid molecules according to  
15 the second aspect of the invention in a host cell and isolating the compound therefrom.

It is preferable that the two portions of the compound of the invention are produced as a fusion compound by recombinant DNA techniques,  
20 whereby a length of DNA comprises respective regions encoding the two portions of the compound of the invention either adjacent one another or separated by a region encoding a linker peptide which does not destroy the desired properties of the compound. The benefits in making the compound of the invention using recombinant DNA techniques are several  
25 fold. Firstly, it enables a high degree of precision with which the two portions of the compound can be joined together. Secondly, the construction of compounds which are "hetero-oligomeric" can be controlled by the expression of the different recombinant DNA molecules

encoding each of the different type of subunit of the "hetero-oligomer" in the same host cell.

By "hetero-oligomer" we mean those compounds in which two or more  
5 different cell-specific portions are joined to either the same or to different subunits which are capable of oligomerisation. The expression, in the same host cell of two compounds, of A and B, each with different target cell specific portions but with a common second portion capable of oligomerisation will result in a mixed population of compounds. For  
10 example, if the common second portion is capable of dimerisation, three potential compounds will be produced:  $A_2$ , AB and  $B_2$ , in a ratio of 1:2:1, respectively.

The separation of the desired compound with each of the different cell  
15 specific portions, that is AB, can be achieved by two step affinity chromatography.

Application of the mixture of compounds to an affinity column specific for A will result in the binding of  $A_2$  and AB. These compounds are eluted  
20 from this first column, and then applied to an affinity column specific for B. This will result in AB, but not  $A_2$ , being bound to the column. Finally, the desired product AB, can be eluted.

Of course, the order in which the affinity columns are used is not  
25 important.

The same principle of separating those compounds with two or more different binding sites can be applied to the purification of the desired

compounds from mixtures of other hetero-oligomers.

Conceivably, the two portions of the compound may overlap wholly or partly.

5

Preferably, the compound is a multimeric compound such as a whole antibody/DNase fusion comprising two light chains and two heavy chains ( $H_2L_2$ ), a  $F(ab')_2$  fusion comprising two light chains and two truncated heavy chains ( $Fd_2L_2$ ), or a Fab fusion comprising one light chain and one  
10 truncated heavy chain ( $FdL$ ).

The nucleic acid may be expressed in a suitable host to produce a polypeptide comprising the compound of the invention. Thus, the nucleic acid encoding the compound of the invention or a portion thereof may be  
15 used in accordance with known techniques, appropriately modified in view of the teachings contained herein, to construct an expression vector, which is then used to transform an appropriate host cell for the expression and production of the polypeptide of the invention. Such techniques include those disclosed in US Patent Nos. 4,440,859 issued 3 April 1984 to Rutter  
20 *et al*, 4,530,901 issued 23 July 1985 to Weissman, 4,582,800 issued 15 April 1986 to Crowl, 4,677,063 issued 30 June 1987 to Mark *et al*, 4,678,751 issued 7 July 1987 to Goeddel, 4,704,362 issued 3 November 1987 to Itakura *et al*, 4,710,463 issued 1 December 1987 to Murray, 4,757,006 issued 12 July 1988 to Toole, Jr. *et al*, 4,766,075 issued 23  
25 August 1988 to Goeddel *et al* and 4,810,648 issued 7 March 1989 to Stalker, all of which are incorporated herein by reference.

Where the compound of the invention is multimeric, the constituent chains

may be encoded by a single nucleic acid molecule or separate nucleic acid molecule (expressed in a common host cell or in different host cells and assembled *in vitro*).

- 5    The nucleic acid encoding the compound of the invention or a portion thereof may be joined to a wide variety of other nucleic acid sequences for introduction into an appropriate host. The companion nucleic acid will depend upon the nature of the host, the manner of the introduction of the nucleic acid into the host, and whether episomal maintenance or  
10   integration is desired.

It will be appreciated that in order to prevent expression of the cytotoxic portion of the compound of the invention from killing the host cells in which it is expressed, it may be necessary to link the nucleic acid of the  
15   second aspect of the invention to a signal sequence capable of directing secretion of the expressed compound (or portion) out of the host cell. Signal sequences will be selected according to the type of host cell used. Exemplary signal sequences include the *ompA* signal sequence (for example, see Takahara *et al.*, 1985, *J. Biol. Chem.* **260**(5):2670-2674).

20

Generally, the nucleic acid is inserted into an expression vector, such as a plasmid, in proper orientation and correct reading frame for expression. If necessary, the nucleic acid may be linked to the appropriate transcriptional and translational regulatory control nucleotide sequences  
25   recognised by the desired host, although such controls are generally available in the expression vector. For example, the nucleic acid molecule encoding a compound of the invention may be linked to or comprise a Kozak consensus ribosome binding sequence (such as GCCGCCACC) to

enhance translation.

The vector is then introduced into the host through standard techniques.

Generally, not all of the hosts will be transformed by the vector.

- 5 Therefore, it will be necessary to select for transformed host cells. One selection technique involves incorporating into the expression vector a nucleic acid sequence, with any necessary control elements, that codes for a selectable trait in the transformed cell, such as antibiotic resistance. Alternatively, the gene for such selectable trait can be on another vector,  
10 which is used to co-transform the desired host cell.

- Host cells that have been transformed by the recombinant nucleic acid of the invention are then cultured for a sufficient time and under appropriate conditions known to those skilled in the art in view of the teachings  
15 disclosed herein to permit the expression of the polypeptide, which can then be recovered.

- Many expression systems are known, including bacteria (for example *E. coli* and *Bacillus subtilis*), yeasts (for example *Saccharomyces cerevisiae*  
20 and *Pichia pastoris*), filamentous fungi (for example *Aspergillus*), plant cells, animal cells (for example COS-1, COS-7, CHO, NIH 3T3, NS0 and BHK cells) and insect cells (for example *Drosophila*, SF9 cells).

- Those vectors that include a replicon such as a procaryotic replicon can  
25 also include an appropriate promoter such as a procaryotic promoter capable of directing the expression (transcription and translation) of the genes in a bacterial host cell, such as *E. coli*, transformed therewith.

A promoter is an expression control element formed by a DNA sequence that permits binding of RNA polymerase and transcription to occur. Promoter sequences compatible with exemplary bacterial hosts are typically provided in plasmid vectors containing convenient restriction  
5 sites for insertion of a DNA segment of the present invention.

Typical procaryotic vector plasmids are pUC18, pUC19, pBR322 and pBR329 (available from Biorad Laboratories, Richmond, CA, USA), pTrc99A and pKK223-3 (available from Pharmacia Piscataway, NJ, USA)  
10 and the pET system (T7 promoter, Novagen Ltd).

A typical mammalian cell vector plasmid is pSVL available from Pharmacia, Piscataway, NJ, USA. This vector uses the SV40 late promoter to drive expression of cloned genes, the highest level of  
15 expression being found in T antigen-producing cells, such as COS-1 cells.

An example of an inducible mammalian expression vector is pMSG, also available from Pharmacia. This vector uses the glucocorticoid-inducible promoter of the mouse mammary tumour virus long terminal repeat to  
20 drive expression of the cloned gene.

Useful yeast plasmid vectors are pRS403-406 and pRS413-416 and are generally available from Stratagene Cloning Systems, La Jolla, CA 92037, USA. Plasmids pRS403, pRS404, pRS405 and pRS406 are Yeast  
25 Integrating plasmids (YIps) and incorporate the yeast selectable markers *his3*, *trp1*, *leu2* and *ura3*. Plasmids pRS413-416 are Yeast Centromere plasmids (YCps).

Further useful vectors for transformation of yeast cells, such as *Pichia*, include the  $2\mu$  plasmid pYX243 (available from R and D Systems Limited) and the integrating vector pPICZ series (available from Invitrogen).

- 5 A variety of methods have been developed to operatively link DNA to vectors via complementary cohesive termini. For instance, complementary homopolymer tracts can be added to the DNA segment to be inserted to the vector DNA. The vector and DNA segment are then joined by hydrogen bonding between the complementary homopolymeric  
10 tails to form recombinant DNA molecules.

- Synthetic linkers containing one or more restriction sites provide an alternative method of joining the DNA segment to vectors. The DNA segment, generated by endonuclease restriction digestion as described  
15 earlier, is treated with bacteriophage T4 DNA polymerase or *E. coli* DNA polymerase I, enzymes that remove protruding, 3'-single-stranded termini with their 3'-5'-exonucleolytic activities, and fill in recessed 3'-ends with their polymerizing activities.

- 20 The combination of these activities therefore generates blunt-ended DNA segments. The blunt-ended segments are then incubated with a large molar excess of linker molecules in the presence of an enzyme that is able to catalyze the ligation of blunt-ended DNA molecules, such as bacteriophage T4 DNA ligase. Thus, the products of the reaction are  
25 DNA segments carrying polymeric linker sequences at their ends. These DNA segments are then cleaved with the appropriate restriction enzyme and ligated to an expression vector that has been cleaved with an enzyme that produces termini compatible with those of the DNA segment.

Synthetic linkers containing a variety of restriction endonuclease sites are commercially available from a number of sources including International Biotechnologies Inc, New Haven, CN, USA.

5

A desirable way to modify the nucleic acid encoding the compound of the invention or a portion thereof is to use the polymerase chain reaction as disclosed by Saiki *et al* (1988) *Science* **239**, 487-491.

10 In this method the nucleic acid to be enzymatically amplified is flanked by two specific oligonucleotide primers which themselves become incorporated into the amplified nucleic acid. The said specific primers may contain restriction endonuclease recognition sites which can be used for cloning into expression vectors using methods known in the art.

15

Exemplary genera of yeast contemplated to be useful in the practice of the present invention are *Pichia*, *Saccharomyces*, *Kluyveromyces*, *Candida*, *Torulopsis*, *Hansenula*, *Schizosaccharomyces*, *Citeromyces*, *Pachysolen*, *Debaromyces*, *Metschnikowia*, *Rhodosporidium*, *Leucosporidium*,  
20 *Botryosascus*, *Sporidiobolus*, *Endomycopsis*, and the like. Preferred genera are those selected from the group consisting of *Pichia*, *Saccharomyces*, *Kluyveromyces*, *Yarrowia* and *Hansenula*. Examples of *Saccharomyces* are *Saccharomyces cerevisiae*, *Saccharomyces italicus* and *Saccharomyces rouxii*. Examples of *Kluyveromyces* are *Kluyveromyces*  
25 *fragilis* and *Kluyveromyces lactis*. Examples of *Hansenula* are *Hansenula polymorpha*, *Hansenula anomala* and *Hansenula capsulata*. *Yarrowia lipolytica* is an example of a suitable *Yarrowia* species.

Methods for the transformation of *S. cerevisiae* are taught generally in EP 251 744, EP 258 067 and WO 90/01063, all of which are incorporated herein by reference.

- 5     Suitable promoters for *S. cerevisiae* include those associated with the *PGK1* gene, *GAL1* or *GAL10* genes, *CYC1*, *PHO5*, *TRP1*, *ADH1*, *ADH2*, the genes for glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, triose phosphate isomerase, phosphoglucose isomerase, glucokinase,  $\alpha$ -mating factor pheromone, a-
- 10     mating factor pheromone, the *PRB1* promoter, the *GUT2* promoter, and hybrid promoters involving hybrids of parts of 5' regulatory regions with parts of 5' regulatory regions of other promoters or with upstream activation sites (e.g. the promoter of EP-A-258 067).
- 15     The transcription termination signal is preferably the 3' flanking sequence of a eukaryotic gene which contains proper signals for transcription termination and polyadenylation. Suitable 3' flanking sequences may, for example, be those of the gene naturally linked to the expression control sequence used, i.e. may correspond to the promoter. Alternatively, they
- 20     may be different in which case the termination signal of the *S. cerevisiae* *AHD1* gene is preferred.

The present invention also relates to a host cell transformed with a polynucleotide vector construct of the present invention. The host cell can

25     be either procaryotic or eukaryotic. Bacterial cells are preferred procaryotic host cells and typically are a strain of *E. coli* such as, for example, the *E. coli* strains DH5 available from Bethesda Research Laboratories Inc., Bethesda, MD, USA, and RR1 available from the

- American Type Culture Collection (ATCC) of Rockville, MD, USA (No ATCC 31343). Preferred eukaryotic host cells include yeast and mammalian cells, preferably vertebrate cells such as those from a mouse, rat, monkey or human fibroblastic cell line. Preferred eukaryotic host
- 5 cells include Chinese hamster ovary (CHO) cells available from the ATCC as CCL61, NIH Swiss mouse embryo cells NIH/3T3 available from the ATCC as CRL 1658 and monkey kidney-derived COS-1 cells available from the ATCC as CRL 1650 or WSØ cells.
- 10 Transformation of appropriate cell hosts with a nucleic acid constructs of the present invention is accomplished by well known methods that typically depend on the type of vector used. With regard to transformation of procaryotic host cells, see, for example, Cohen *et al*, *Proc. Natl. Acad. Sci. USA*, **69**: 2110 (1972); and Sambrook *et al*,
- 15 *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1989). Transformation of yeast cells is described in Sherman *et al*, *Methods In Yeast Genetics, A Laboratory Manual*, Cold Spring Harbor, NY (1986). The method of Beggs, *Nature*, **275**: 104-109 (1978) is also useful. With regard to
- 20 vertebrate cells, reagents useful in transfecting such cells, for example calcium phosphate and DEAE-dextran or liposome formulations, are available from Stratagene Cloning Systems, or Life Technologies Inc, Gaithersburg, MD 20877, USA.
- 25 Successfully transformed cells, *i.e.* cells that contain a nucleic acid construct of the present invention, can be identified by well known techniques. For example, cells resulting from the introduction of an expression construct of the present invention can be grown to produce the

polypeptide of the invention. Cells can be harvested and lysed and their DNA content examined for the presence of the DNA using a method such as that described by Southern, *J. Mol. Biol.*, **98**: 503 (1975) or Berent *et al*, *Biotech.*, **3**: 208 (1985). Alternatively, the presence of the protein in  
5 the supernatant can be detected using antibodies as described below.

In addition to directly assaying for the presence of recombinant nucleic acid, successful transformation can be confirmed by well known immunological methods when the recombinant nucleic acid is capable of  
10 directing the expression of the protein. For example, cells successfully transformed with an expression vector produce proteins displaying appropriate antigenicity. Samples of cells suspected of being transformed are harvested and assayed for the protein using suitable antibodies.

15 Thus, in addition to the transformed host cells themselves, the present invention also contemplates a culture of those cells, preferably a monoclonal (clonally homogeneous) culture, or a culture derived from a monoclonal culture, in a nutrient medium. Preferably, the culture also contains the protein.

20

Nutrient media useful for culturing transformed host cells are well known in the art and can be obtained from several commercial sources.

A third aspect of the invention provides a vector comprising a nucleic acid  
25 according to the second aspect of the invention.

A fourth aspect of the invention provides a host cell comprising a vector according to the third aspect of the invention.

Preferably, the host cell is a mammalian cell.

More preferably the host cell is NS0 or CHO.

5

A fifth aspect of the invention provides a pharmaceutical composition comprising a compound according to the first aspect of the invention and a pharmaceutically acceptable carrier.

- 10 The compounds and compositions of the invention are administered in any suitable way, usually parenterally, for example intravenously, intraperitoneally or, preferably (for bladder cancer), intravesically (*i.e.* into the bladder), in standard sterile, non-pyrogenic formulations of diluents and carriers, for example isotonic saline (when administered
- 15 intravenously).

A sixth aspect of the invention provides a compound according to the first aspect of the invention for use in medicine.

- 20 The compounds and compositions of the invention may be used to treat a patient with any disease involving a dysfunction of a population of cells expressing PEM, said compounds and compositions selectively targeting and destroying said population of cells within a patient. For example, said compounds and compositions may be used in the treatment of cancer, *e.g.*
- 25 cancer of the breast, ovaries, lung, stomach, intestines, blood *etc.* Thus, anti-tumour cell antigen antibodies can be used to deliver a cytotoxic portion with endonuclease activity to a tumour cell. Antibodies that are internalised upon contact with the target antigen are used, such that the

cytotoxic portion enters the cytosol of the tumour cell, where it can trigger cell death.

In principle, the compounds and compositions of the invention may be  
5 used to treat any mammal, including pets such as dogs and cats and agriculturally important animals such as cows, horses, sheep and pigs.

Preferably, the patient is human.

10 A seventh aspect of the invention provides the use of a compound according to first aspect of the invention in the preparation of a medicament for treating a mammal having said target cells to be destroyed.

15 Preferably, the medicament is for treating cancer, such as ovarian cancer.

A eighth aspect of the invention provides a method of treating a mammal having target cells to be destroyed, the method comprising administering a compound according to the first aspect of the invention to said mammal.

20

In a preferred embodiment of the seventh and eighth aspects of the invention, the mammal is a human.

Preferably, the target cells to be destroyed are cancer cells. More  
25 preferably, the cancer cells are epithelial cancer cells, such as ovarian, gastric, colorectal and/or pancreatic cancer cells. Most preferably, the cancer cells are ovarian cancer cells.

The invention will now be described in detail with reference to the following figures and examples:

Figure 1 shows the complete coding sequence of human DNase I.

5

Figure 2 shows (A) the mature DNase peptide I sequence used in the exemplary Ab-DNase and Fab-DNase constructs, and (B) a truncated DNase peptide I sequence encoded by a nucleotide sequence comprising a Kozak sequence (underlined).

10

Figure 3 shows (A) the nucleotide sequence encoding the humanised HMFG1 light chain including leader peptide, (B) the nucleotide sequence of (A) further comprising a Kozak sequence (underlined), (C) the amino acid sequence of the humanised HMFG1 light chain including leader peptide (shaded) and (D) the nucleotide sequence encoding the humanised HMFG1 heavy chain including leader peptide,

15

Figure 4 shows the linker and hinge-linker oligonucleotides used in (A) the whole antibody-DNase and (B) the Fd-DNase exemplary constructs.

20 Note, in Figure 4(A) a deletion of one or more codons between the HMFG1 hinge and the linker is represented as  $\Delta$ G.

Figure 5 shows nucleotide sequences (A and B) encoding a humanised HMFG-1 Fd/DNase I fusion pAS23 comprising a leader sequence (underlined) and a linker sequence (double-underlined). Figure 5(C) shows the nucleotide sequence of (B) further comprising a Kozak sequence (underlined). Figure (D) shows the amino acid sequence of a humanised HMFG-1 Fd/DNase I fusion.

25

Figure 6 shows (A), (B) and (C) shows the nucleotide sequences of Figure 5 (A), (B) and (C), respectively, further comprising an SV40 NLS (double underlined) (pAS27). Figure (D) shows the amino acid sequence of a humanised HMFG-1 Fd/DNase I fusion comprising an SV40 NLS (double underlined).

Figure 7 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of an exemplary HMFG-1 heavy chain/DNase I fusion pAS34 (as used in 'Ab-DNase' in Example 2), comprising a leader sequence (underlined) and a linker sequence (double-underlined).

Figure 8 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of an exemplary HMFG-1 heavy chain/DNase I fusion pAS35, comprising a leader sequence (underlined) and a linker sequence (double-underlined). The lower case 'g' represents a silent mutation caused by PCR amplification.

Figure 9 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of an exemplary HMFG-1 heavy chain/DNase I fusion pAS36, comprising a leader sequence (underlined) and a linker sequence (double-underlined). The lower case 'c' represents a silent mutation caused by PCR amplification.

Figure 10 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of an exemplary HMFG-1 heavy chain/DNase I fusion pAS37, comprising a leader sequence (underlined), a linker sequence (double-underlined) and an NLS sequence (triple underlined).

Figure 11 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of an exemplary HMFG-1 heavy chain/DNase I fusion pAS38, comprising a leader sequence (underlined), a linker sequence (double-underlined) and an NLS sequence (triple underlined). The lower case 'g' represents a silent mutation caused by PCR amplification.

Figure 12 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of an exemplary HMFG-1 heavy chain/DNase I fusion pAS39, comprising a leader sequence (underlined), a linker sequence (double-underlined) and an NLS sequence (triple underlined). The lower case 'c' represents a silent mutation caused by PCR amplification.

Figure 13 shows nucleotide sequences (A and B) encoding a humanised HMFG-1 Fd/DNase I fusion pAS101 comprising a short leader sequence (underlined) and a linker sequence (double-underlined). Figure 13(C) shows the nucleotide sequence of (B) further comprising a Kozak sequence (underlined). Figure (D) shows the amino acid sequence of a humanised HMFG-1 Fd/DNase I fusion.

20

Figure 14 shows nucleotide sequences (A and B) encoding a humanised HMFG-1 Fd/DNase I fusion pAS102 comprising a leader sequence (underlined) and a hybrid hinge + linker sequence (double-underlined).

Figure 14(C) shows the nucleotide sequence of (B) further comprising a Kozak sequence (underlined) (construct designated pAS302 in Example 2).

Figure (D) shows the amino acid sequence of a humanised HMFG-1 Fd/DNase I fusion.

Figure 15 shows nucleotide sequences (A and B) encoding a humanised HMFG-1 Fd/DNase I fusion pAS103 comprising a leader sequence (underlined) and a hybrid hinge + short linker sequence (double-underlined). Figure 15(C) shows the nucleotide sequence of (B) further comprising a Kozak sequence (underlined). Figure (D) shows the amino acid sequence of a humanised HMFG-1 Fd/DNase I fusion.

Figure 16 shows nucleotide sequences (A and B) encoding a humanised HMFG-1 Fd/DNase I fusion pAS104 comprising a leader sequence (underlined) and a hybrid hinge + mutated short linker sequence (double-underlined). Figure (C) shows the amino acid sequence of a humanised HMFG-1 Fd/DNase I fusion. Mutations (compared to pAS103) at positions 775 and 924 are shaded.

Figure 17 shows nucleotide sequences (A and B) encoding a humanised HMFG-1 Fd/DNase I fusion pAS105 comprising a leader sequence (underlined), a short linker sequence (double-underlined) and an NLS sequence (triple underlined). Figure 17(C) shows the nucleotide sequence of (B) further comprising a Kozak sequence (underlined). Figure (D) shows the amino acid sequence of a humanised HMFG-1 Fd/DNase I fusion.

Figure 18 shows nucleotide sequences (A and B) encoding a humanised HMFG-1 Fd/DNase I fusion pAS106 comprising a leader sequence (underlined), a hybrid hinge + linker sequence (double-underlined) and an NLS sequence (triple underlined). Figure 18(C) shows the nucleotide sequence of (B) further comprising a Kozak sequence (underlined). Figure (D) shows the amino acid sequence of a humanised HMFG-1

Fd/DNase I fusion.

Figure 19 shows nucleotide sequences (A and B) encoding a humanised HMFG-1 Fd/DNase I fusion pAS107 comprising a leader sequence  
5 (underlined), a hybrid hinge + short linker sequence (double-underlined) and an NLS sequence (triple underlined). Figure 19(C) shows the nucleotide sequence of (B) further comprising a Kozak sequence (underlined). Figure (D) shows the amino acid sequence of a humanised HMFG-1 Fd/DNase I fusion.

10

Figure 20 shows a schematic diagram of the pEE6 expression vector used in the exemplary constructs.

Figure 21 shows autoradiographs from immuno-precipitation experiments  
15 with metabolically labelled transient transfectants:

#### **GEL A**

Lane 1 shows the precipitation of supernatant from mock-transfected cells.

20 Lane 2 is from cells transfected with hHMFG-1 (construct 6) giving expected molecular weights of about 51.2 and 26.4 kDa for the heavy and light chains, respectively.

Lane 3 shows construct 34 antibody construct which has human DNase I fused to the C-terminus of the heavy chain gene. As  
25 expected, the size of the heavy chain gene has increased to about 80.7 kDa.

Samples from whole antibody DNase I constructs 35, 36 and 39 were run on the gel (Lanes 4 to 6) but were not sufficiently well

expressed to be visible, in this experiment.

In subsequent experiments using this method, construct 39 was detectable but weak, and constructs 35 and 36 were detectable but very weak. Constructs 37 and 38 have not been tested in this assay system.

Lanes 8 to 10 are fusion of humanised HMFG1 F(ab')<sub>2</sub> with human DNase I (constructs 41, 23 and 102, respectively). F(ab')<sub>2</sub> alone was included in this set of experiments (lane 7, construct 41) but did not express, this was included in later experiments (see gels C and D).

In addition to the light chain (about 26.4 kDa) and the Fd-DNase I fusion (about 56.6 kDa), a third major band is observed at around 40 kDa. Interestingly, this band is observed in the humanised HMFG-1 fusions but not in the antibody alone. Since an anti-F(ab')<sub>2</sub> antibody was used for immuno-precipitation, it is unlikely that this can be proteolysis between immunoglobulin and DNase I sequence.

It probably represents a population of polypeptide produced by premature transcriptional termination (due to DNase I sequence in the 3'-end of the fusion mRNA).

## GEL B

This is the non-reducing gel counterpart to gel A, described above.

Lane 1 is the mock-transfected control cells and lanes 2 and 3 are from the cells transfected with humanised HMFG1 alone (construct 6) and the humanised HMFG-1 fused at the C-terminus to human DNase I, respectively. As before, lanes 4 to 6 are from cell supernatants from cells transfected with constructs 35, 36 and 39.

The gel shows that both the whole antibody and the antibody-DNase I fusion are assembled, with the DNase fusion giving a higher

molecular weight compared to the antibody alone.

Figure 22 shows a typical standard curve used to determine the concentration of PDTRP-binding material in the supernatants of transiently transfected L761h cells. Each point on the curve has been determined twice.

Figure 23 shows typical standard curves used to determine the concentration of bovine DNase I.

10

Figure 24 shows corrected DNase I activity in transiently expressed humanised HMFG1 whole antibody-human DNase I fusions (*i.e.* pAS34, pAS34, pAS35 and pAS6[control]).

15 Figure 25 shows the corrected DNase I activity in transiently expressed humanised HMFG1 F(ab')<sub>2</sub>-human DNase I fusions (*i.e.* pAS101, pAS102, pAS103 and pAS41[control]).

Figure 26 shows results of the cytotoxicity assay.

20

Figure 27 shows the % of MCF7 cells killed after incubation with the exemplary constructs.

Figure 28 shows a schematic diagram of (A) Ab-DNase and (B) Fab-DNase.

25

Figure 29 shows a schematic diagram of vector pAS34K encoding Ab-DNase (*i.e.* pAS34 as shown in Figure 7b plus Kozak sequence).

Figure 30 shows a schematic diagram of vector pAS302 encoding Fab-DNase.

- 5    Figure 31 shows (A) the elution profile from Protein-L column and (B) size exclusion chromatogram for Fab-DNase.

Figure 32 shows (A) the elution profile from Protein-A column and (B) size exclusion chromatogram for Ab-DNase.

10

Figure 33 shows the SDS-PAGE stained gels for (A) Ab-DNase and (B) Fab-DNase.

- Figure 34 shows (A) standard curve for bovine DNase concentration AND  
15    (B) DNase activity measurements at 3 hours and 6 hours.

Figure 35 shows (A) PEM expression on OVCAR 3 and A375 cells, as measured by ELISA using hHMFG-1 and AD-DNase antibodies, and (B) cytotoxicity measurements.

20

## EXAMPLES

### Example 1

#### 5 (A) Mammalian expression of humanised HMFG1-DNase constructs

The human HMFG1 light and heavy chain (with or without engineering a fusion to human DNase I), were cloned into the pEE6 expression vector system for expression in mammalian CHO or myeloid NS0 cells (see  
10 figure 20). The vector system was originally developed by Celltech Ltd (UK) and is now owned by al-Lonza (see Young & Owens, 1994, *J. Immunol. Meth.* **168**:149-165). The vector consists of two human cytomegalovirus promoters (hCMV) for both the heavy and light chain genes. Each transcription unit is completed by the poly-adenylation signal  
15 (pA) with an optional immunoglobulin terminator sequence (Ig term.) located between the heavy and light chain transcription units. Propagation in *E.coli* can be selected for by the presence on an ampicillin resistance gene (not shown in Fig 20). The inclusion of a glutamine synthetase gene (GS) in the vector allows the stable NS0 transfectomas to be selected by  
20 growth in glutamine free media, since NS0 cells are GS<sup>-</sup> and cannot otherwise grow in glutamine free media.

Exemplary humanized HMFG1-DNase I fusion constructs of the invention are detailed in figures 5 to 19.

25

#### (B) Immuno-precipitation of metabolically labelled transient transfectants

CHO-L761h cells (Cockett *et al.*, 1990, *Nuc. Acids Res.* **19**:319-325)

were transfected, according to the modification of Gorman et al, 1985), with expression vectors containing either whole HMFG1 antibody or F(ab')<sub>2</sub> fragment of the antibody along with the various fusion constructs of their respective heavy chains and human DNase I. The cells were then  
5 incubated with either 50  $\mu$ Ci <sup>35</sup>S methione for 72 h in methionine-free medium. Secreted product was precipitated with a rabbit anti-human F(ab')<sub>2</sub> antibody bound to protein A Sepharose. Bound material was eluted in either reducing or non-reducing SDS-PAGE loading buffer and run on gels. The autoradiographs (see Figure 21) above were generated  
10 from those gels after drying them.

(C) Estimation of the efficiency of DNase constructs in supernatants

*Introduction*

15

This set of experiments was designed to standardise the amount of construct in a given DNase I activity assay and to allow us to comment on the amount of activity a particular construct possesses. Given that the antibody-DNase I fusions are so different to the F(ab')<sub>2</sub>-DNase I fusions  
20 it is best not to compare the two groups. Once we have purified the protein, we will have a better idea of the exact molecular configuration of all species. Then, and only then, will it be sensible to compare amongst groups.

25 *Determination of concentration of constructs*

The concentration of constructs in supernatants from transiently transfected L761H cells was determined in a PDTRP-binding ELISA. To

each well of a Maxisorb 96-well ELISA plate (Nunc) was added 100  $\mu$ l of carbonate buffer containing 100 ng of recombinant GST-(PDTRP)<sub>7</sub> fusion protein (Gendler *et al.*, 1990, *J. Mol. Biol.* **265**:15286-93). After overnight binding at 4°C, the plate was washed three times in PBS-Tween  
5 (*i.e.* PBS containing 0.05% Tween-20). The plate was then blocked with three 3-minute washes of PBS-Tween containing 1% BSA.

For each construct, 100  $\mu$ l of supernatant was added to a well on the plate. In addition, hHMFG-1 of known concentration was serially diluted down  
10 the plate using doubling dilutions in 100  $\mu$ l of PBS-Tween per well. The plate was incubated for a further 1 h at 30°C, then 200 ng of MC135 anti-human kappa light chain antibody (binding site) in 100  $\mu$ l of PBS-Tween was added to each well for 1 h at 30°C. After three 3-minute washes in  
15 PBS-Tween, 100  $\mu$ l of anti-mouse IgG-peroxidase conjugate (Jackson 315-035-045), diluted 1:2000 in PBS-Tween, was added to each well and incubated for 1 h at 30°C. Following a final set of three 3-minute washes in PBS-Tween, 100  $\mu$ l of TMB substrate (Sigma) was added to each well of the plate and, after a colour developed, the optical density at 630 nm of the solution in each well of the plate was determined.

20

### *Results*

(see Figure 22)

#### 25 (D) Corrected bovine DNase I standard curves and DNase assay

DNase activity was determined using a modification of the methyl green-DNA complex degradation method (Sinicropi *et al.*, 1994, *Analyt.*

*Biochem.* **222**:351-358). Briefly, a 1:1 solution of the assay buffer and methyl green-salmon sperm DNA complex was mixed together to give a total volume of 0.2 ml. To this, 0.1 ml of tissue culture supernatant from transiently transfected CHO-L761h cells was added and the mixture  
5 incubated at 37°C. DNA cleavage by DNase results in a reduction in absorbance at 620 nm. Figure 23 shows a standard curve produced with various concentrations of bovine DNase I over a number a time point.

Figures 24 and 25 show DNase activity for the whole HMFG1 antibody-  
10 and F(ab')<sub>2</sub> - DNase fusions, respectively.

#### (E) Cytotoxicity of DNase constructs

##### *Method*

15

DNase constructs were transfected into CHO L761h cells using a calcium phosphate co-precipitation method (Gorman *et al.*, 1985, In: *DNA cloning* (2nd edition), Glover A(ed.), Academic Press, NY, 163-188). Included in the experiment were negative controls, consisting of cells transfected  
20 with TE buffer alone or with TE buffer and pEE6 expression vector. In addition to these controls, vectors that express hHMFG-1 (pAS6) and F(ab')<sub>2</sub> of hHMFG1 (both with specificity for PEM but without DNase I) were included.

25 The supernatant from these cells was harvested after 72 h of expression, followed by centrifugation to remove dead cells. MCF-7 cells were incubated for 1 h at 37°C with an aliquot of each of these supernatants. The amount of cellular lactate dehydrogenase (LDH) released from the

MCF-7 cells due to the cytotoxicity of the supernatant was determined using the CytoTox96 cytotoxic assay kit (Promega). Total lysis ('total LDH') was determined by measuring the target cell maximum LDH release using the kits lysis solution. The percentage of cells killed was then calculated as the proportion of the LDH released to the total LDH released. For each construct, the cytotoxicity assay was performed in quadruplicate, except for assay of pAS38 and 39, which were performed in triplicate. The values of LDH release for each construct were compared against either F(ab')<sub>2</sub> or whole antibody, or each other, using a one-tailed t-test in Excel.

### *Results*

Figures 26 and 27 shows that there is negligible cell killing with either pAS6 (HMFG1 alone) or with pAS41 (F(ab')<sub>2</sub> alone). All of the hHMFG1 F(ab')<sub>2</sub>-DNase I constructs kill significantly more cells than the F(ab')<sub>2</sub> fragment alone ( $p < 0.00193$ ) and all of the antibody-DNase I constructs kill significantly more cells than antibody alone ( $p < 0.00783$ ), except for perhaps pAS34 ( $p < 0.021$ ).

### (F) Use of the DNase-I/huHMFG-1 Fab fusion protein in the treatment of ovarian cancer

Patients diagnosed with ovarian cancer are treated by intravenous injection of the DNaseI/huHMFG-1 Fab fusion protein. Typically, a dose of between 1 to 100 mg will be administered weekly.

Therapeutic response is measured by the normal clinical procedures that

are well known in the art, for example radio-imaging methods.

**Example 2**

5 (A) Mammalian expression of humanised HMFG-1 / DNase constructs

In a second series of experiments, two further humanised HMFG-1/Dnase constructs were expressed in mammalian cells. The first construct encoded a fusion protein a complete hHMFG-1 antibody fused with human  
10 DNase, designated 'Ad-DNase'. The second construct encoded a fusion protein a Fab fragment of the hHMFG-1 antibody fused with human DNase, designated 'Fab-DNase'. Ad-Dnase and Fab-DNase are shown schematically in Figure 28.

15 Ad-DNase comprises an HMFG-1 light chain as shown in Figure 3(c) and an HMFG-1 heavy chain/DNase fusion as shown in Figure 7(b).

Fab-DNase comprises an HMFG-1 light chain as shown in Figure 3(c) and an HMFG-1 Fd chain/DNase fusion as shown in Figure 14(d).

20

The human HMFG1 heavy and light chain constructs were cloned into the pEE6 expression vector system for expression in mammalian CHO or myeloid NS0 cells, as described in Section (A) of Example 1. This vector consists of two human cytomegalovirus promoters (hCMV) for both the  
25 heavy and light chain genes. Each transcription unit is completed by the poly-adenylation signal (pA) with an optional immunoglobulin terminator sequence (Ig term.) located between the heavy and light chain transcription units. The vectors also comprise a 5'-UT Kozak sequence

(to enhance translation of the mRNA) and an ATG initiator codon upstream of both heavy and light chains.

The vectors encoding Ad-Dnase and Fab-DNase, designated pAS34K and  
5 pAS 302 respectively, are shown schematically in Figure 32.

Propagation in *E.coli* can be selected for by the presence on an ampicillin resistance gene. The inclusion of a glutamine synthetase gene (GS) in the vector allows the stable NS0 transfectomas to be selected by growth in  
10 glutamine free media, since NS0 cells are GS<sup>-</sup> and cannot otherwise grow in glutamine free media.

These plasmids were co-transfected with a vector containing a neomycin resistance gene into CHO cells. Stable cell lines were generated for each  
15 of the constructs.

Clones were selected that expressed DNase activity and antigen (PEM)-binding activity.

20 (B) Purification of hHMFG-1/DNase constructs

The cells were routinely grown in:

	DMEM (Gibco 10938-025)	500 ml
25	Non essential amino acids (Sigma M7145)	5 ml
	Sodium pyruvate (Sigma S8636)	5 ml
	Glutamine (G7513)	5 ml
	Heat inactivated foetal calf serum	50 ml

Incubation was carried out at 37°C in 5% CO<sub>2</sub>.

For production of the Ab-DNase fusion protein, W70 cells (CHO cells  
5 transfected with pAS34K) were maintained in flats and grown to  
confluency in T175 flasks. Each T175 flask was split between two  
850 cm<sup>2</sup> roller bottles containing 100 ml of the aforementioned growth  
media. Each roller bottle was gassed with an 95% air 5% CO<sub>2</sub> mix for 1  
minute and then sealed. They were rolled at a rate of 0.5 rpm and were  
10 gassed every other day as described earlier until the cultures were  
confluent. At this stage the medium was removed and 200 ml of harvest  
medium was replaced on the culture. This was the same medium but  
contained 2 mM sodium butyrate (with or without 10% heat inactivated  
FCS). The cells were then grown for a further 3-4 days before they were  
15 harvested. The medium was collected from the cells and dead cells were  
removed from the medium by centrifugation at 5000 rpm for 30 mins at  
4°C. The spun medium (supernatant) was then filtered through a 0.2  
micron filter unit, prior to applying to the affinity chromatography  
column.

20

The Fab-DNase fusion product was then purified by affinity  
chromatography using a Protein-L column (Protein L agarose, P3351 from  
Sigma Co, Poole, Dorset, UK), as follows:

- 25
1. Wash 1 ml of settled protein L agarose (P3351) with at least 5  
volumes of phosphate buffered saline (PBS: 10 mM phosphate  
buffered saline, pH 7.4).
  2. Dilute 1 ml supernatant with 9 ml PBS.

3. Mix diluted supernatant with protein-L agarose and incubate with gentle end over end mixing for 1 hour at room temperature.
4. Pack the slurry in a column and drain.
5. Wash away unbound proteins with 10-15 column volumes of PBS.
- 5 6. Elute bound protein with 5 ml elution buffer (0.1 M glycine, pH 2.0, or 0.2 M citrate buffer, pH 2.8).
7. Neutralise eluted material with Tris-base to achieve pH 7.5.

Figure 31(a) shows the elution profile of the Fab-DNase from the Protein-L column when eluted with 0.1 M glycine, pH 2.0.

Following purification, Fab-DNase was analysed by analytical size-exclusion chromatography on a Superdex-200 column.

15 Figure 31(b) shows the size-exclusion chromatogram obtained for the Fab-DNase.

The Ab-DNase fusion product was purified by affinity chromatography using a Protein-A sepharose column, as follows:

20

1. 25 ml of protein A sepharose fast flow resin (Amersham Pharmacia Biotech) in an XK26 column (Amersham Pharmacia Biotech) was equilibrated in 0.1M glycine, pH 8.8, 0.5M NaCl.
2. Approximately 2 litres of sterile-filtered supernatant from cell line  
25 W70 (CHO cell line making 34K) was passed the column overnight at a low flow rate (1-2 ml/min).
3. The column was then washed down to base-line and was re-equilibrated in 0.15M disodium hydrogen phosphate, pH 9.0 and the

- bound 34K was eluted by running a gradient between this buffer (A) and a low pH buffer (B) which consisted of 0.1M citric acid, pH2.0, supplemented to 2 mM calcium chloride and 2 mM magnesium sulphate. The gradient was run over 100 ml at a flow rate of 4 ml/min and a further 50 ml of buffer B was run over the column at the completion of the gradient, also at 4 ml/min.
- 5
4. During the 100 ml gradient and the last 50 ml of buffer A fractions were collected. The peak fractions were identified and pooled and dialysed against 4 litres of 25 mM Hepes, pH7.5, 0.2 M NaCl, 1mM calcium chloride and 1mM magnesium sulphate. Dialysis was performed overnight at 4C.
- 10
5. The dialysate was concentrated on Centricon spin concentrators to a final concentration of 6-13 mg/ml. The concentration was determined by dividing by its extinction coefficient of 1.558 (calculated from the known sequence).
- 15

Figure 32(a) shows the elution profile of the Ab-DNase from the Protein-L column when eluted with a gradient of 0.15 M  $\text{Na}_2\text{HPO}_4$ , pH 9.0 to 0.1 M citric acid, pH 2.0 containing 2mM each of  $\text{CaCl}_2$  and  $\text{MgCl}_2$ .

20

Figure 32(b) shows the size-exclusion chromatogram obtained for the Ab-DNase.

#### (C) Determination of concentration of fusion proteins

25

Prior to measuring DNase activity of the purified fusion proteins (see Section (E) below), the concentration of the proteins was determined by ELISA, as follows (see also Section (C) of Example 1).

### *Materials*

1. 96 Well ELISA plates (Nunc F96 Maxisorp Cat No. 442404).
- 5      2. Bovine serum albumin (Sigma A-9647).
3. Coating buffer (Na<sub>2</sub>CO<sub>3</sub> 1.59 g/l, NaHCO<sub>3</sub> 2.93 g/l, NaN<sub>3</sub> 0.2 g/l, pH9.6).
4. GST-MUC1-7TR antigen (1.5 mg/ml).
5. Anti-human kappa light-chain antibody GD12 (0.2 mg/ml, Binding Site, MC135).
- 10      6. Peroxidase-conjugated rabbit anti-mouse IgG (Jackson, 315-035-045).
7. TMB- substrate buffer (Sigma P-4417).
8. Tween 20 (Sigma P7949).
- 15      9. Purified humanised HMFG1 (1.4 mg/ml).

### *Method*

Note all washes in this protocol consist of 3 x 3 min washes in PBS buffer  
20 (note: all PBS buffer contained 0.05 % Tween) and the plate was incubated in a lunch box containing moist tissue paper.

1. Coat 100 ng of antigen/100  $\mu$ l coating buffer/well overnight at 4°C.
- 25      2. Wash the plate and block each well with 100  $\mu$ l of PBS containing 0.05 % Tween, and 1 % BSA for 1 h at 30°C. Wash plate afterwards.
3. A standard curve of humanised HMFG1 should be prepared

down the plate using doubling dilutions. Make each dilution in 100  $\mu$ l PBS buffer and for the highest concentration in the curve use 1000 ng of antibody.

- 5           4. Incubate the plate for 2 h at 30°C, wash, and add 100  $\mu$ l PBS containing 200 ng of the anti-human Kappa light chain antibody to each well of the plate. Incubate for a further 1 h at 30°C and then wash the plate.
- 10          5. Add 100  $\mu$ l PBS containing the rabbit anti-mouse IgG-peroxidase conjugate (diluted 1:2000) to each well of the plate and incubate for 30 min at 30°C. Wash the plate and add 100  $\mu$ l TMB- substrate-buffer to each well of the plate and allow the reaction to proceed in the dark at room temperature. When the blue colour has developed, read the plate at a wavelength of 630 nm.

15

#### (D) SDS-PAGE

Following purification of Ab-DNase and Fab-DNase, the fusion proteins were analysed by SDS-PAGE under non-reducing and reducing  
20 conditions, as described in Section (B) of Example 1.

In brief, affinity-purified material was used. In the case of the Ab-DNase fusion protein, this was from a sample dialysed and concentrated (as described in the protein A protocol above). In the case of the Fab-DNase,  
25 this was unconcentrated protein directly eluted from the protein L affinity column. 15  $\mu$ l of the Fab-DNase protein-L eluate was mixed with 5  $\mu$ l of either reducing or non-reducing loading buffer whereas 2  $\mu$ l of the Ab-DNase protein A eluate (dialysed and concentrated) was mixed with 5  $\mu$ l

of either reducing or non-reducing buffer. Both samples were boiled for 5 minutes and were loaded onto the gel. The gels were stained with Coomassie Brilliant Blue stain. The cells were not labelled with 35S-methionine (as in Example 1).

5

The SDS-PAGE autoradiograph for Ab-DNase is shown in Figure 33(a). Under reducing conditions, Ab-DNase produces a band of about 80 kDa, which corresponds to the expected size of the heavy chain-DNase fusion product (see lane 3). A further band of about 50 kDa is also observed,  
10 which is approximately the same molecular weight as the hHMFG-1 heavy chain (see lane 4).

The SDS-PAGE autoradiograph for Fab-DNase is shown in Figure 33(b). Under reducing conditions, Fab-DNase produces a band of about 55-  
15 60 kDa, which corresponds to the expected size of Fab-DNase (see lane 3). Under non-reducing conditions, a band of about 80-85 kDa is observed, which is the approximate molecular weight of Fab-DNase rather than  $F(ab')_2$ -DNase (see lane 4). Thus, the Fab-DNase appears to exist as a dimer of the hHMFG-1 light chains and the hHMFG-1 heavy  
20 chain/human DNase fusion, not a tetrameric  $F(ab')_2$ -DNase.

(E) Measurement of DNase activity of hHMFG-1/DNase constructs

DNase activity of the two fusion proteins was determined as described in  
25 Section (D) of Example 1. In brief, 0.1 ml of the purified protein was added to a 1:1 solution of assay buffer and methyl green-salmon sperm DNA complex, and the mixture incubated at 37°C. A reduction in absorbance at 620 nm is indicative of DNA activity.

A standard curve produced using bovine DNase I is shown in Figure 34(a).

- 5 Figure 34(b) shows the DNase activity of the Fab-DNase and Ab-DNase fusion proteins 3 h and 6 h after being added to the DNA, compared to a positive control of bovine DNase and a negative control of Fab only. Clearly, the DNase activity of the Fab-DNase and Ab-DNase fusion proteins is comparable to that of the bovine DNase positive control.

10

(F) Cytotoxicity of DNase activity of hHMFG-1/DNase constructs

- Cytotoxicity of the Fab-DNase and Ab-DNase fusion proteins was analysed using two tumour cell lines, the human malignant melanoma cell  
15 line A375 and the human ovarian adenocarcinoma cell line OVCAR 3.

An initial cell-based ELISA was performed using hHMFG-1 antibodies to determine the level of expression of PEM (the MUC1 gene product) on these cells.

20

*Cell-based PEM ELISA assay protocol*

Materials and methods

- 25
1. Phosphate buffered saline tablets (Sigma P-4417)
  2. 50% glutaraldehyde solution (BDH UN2810 Prod. 2868240)
  3. sodium azide (Sigma S-8032)
  4. Nunclon 96 well tissue culture plate (Nunc D167008)

5. BSA (Sigma A-9647)
6. OVCAR-3 ovarian cancer cells, A375 melanoma cancer cells both from ATCC
7. TMB substrate buffer (Sigma P-4417)
- 5 8. Tween 20 (Sigma P7949)
9. Purified humanised HMFG1 (1 mg/ml from ICRF)
10. RPMI 1640 media (Gibco 21875-034)

### Protocol

- 10 1. The OVCAR-3 and A375 cells were grown in RPMI containing 20% and 10% FCS respectively at 37°C in 5% CO<sub>2</sub> in a 96 well tissue culture plate, seeded at 10<sup>6</sup> cells/ml with 0.1 ml/well.
2. Excess media was removed and the plate was fixed with 0.05% glutaraldehyde in water for 1 hour at room temperature.
- 15 3. Excess glutaraldehyde/water solution was removed and the plates were washed three times with PBS containing 0.05% Tween 20. The plate was stored at 4°C until required in PBS with 0.02% sodium azide).
- 20 4. To use the plate, the plate was then washed with three washes of PBS containing 0.05% Tween 20, and the wells were blocked with 0.1 ml 5% BSA in PBS containing 0.05% Tween 20. The wells were blocked for 1 hour at 30°C.
- 25 5. They washed three times as described before. Serial dilutions of hHMFG1 were plated out on the wells from a maximum concentration of 2 µg/ml downward. Dilutions of constructs were also similarly plated onto the fixed cells. All dilutions were prepared in PBS containing 0.05% Tween 20.

6. The proteins were incubated with the fixed cells for 1 hour at 30°C and were again washed three times as described above.
7. Anti-human IgG-Fc peroxidase conjugate antibody (Jackson 209-035-103) was diluted to 1:2000 in PBS containing 0.05% Tween  
5 20. This was incubated at 30°C for 30 minutes.
8. Once again the cells were washed as described as before. Then 0.1 ml TMB substrate was put in each well and the colour was developed at room temperature and the absorbance at 655 nm was determined.

10

For comparison, an additional ELISA using Ab-DNase was performed with the OVCAR 3 cells.

15

Antigen-bound hHMFG-1 and Ab-DNase was detected by a peroxidase-conjugated anti-human Fc antibody.

The results of the ELISA are shown in Figure 35, indicating that the OVCAR 3 cell line expresses high levels of PEM (as measured by both hHMFG-1 and Ab-DNase) while the A375 cell line expresses low levels  
20 of PEM (and hence can be used as a negative control in cytotoxicity experiments).

Cytotoxicity was measured using an LDH release assay, as described in Section (E) of Example 1. In brief,  $10^5$  cells per well of the A375 and  
25 OVCAR 3 cell lines were plated in a 96-well plate and grown for 24 hours. Fifteen microlitres of the purified fusion proteins (containing 200 ng of Ab-DNase or 100 ng of Fab-DNase) were added to the cells and incubated for 48 hours at 37°C. A negative control group of each cell

type was treated with 200 ng of the hHMFG-1 antibody (*i.e.* not fused to DNase).

5      Following the incubation period, 50  $\mu$ l of the supernatant was removed  
and incubated with 50  $\mu$ l of tetrazolium-containing substrate buffer for 30  
minutes at 22°C. The reaction was stopped with stop buffer (Promega)  
and the absorbance of the reaction mixture at 490 nm measured.

10      Both Fab-DNase and Ab-DNase fusions show cell killing of OVCAR 3  
cells as compared to the negative control hHMFG-1 treated cells. In  
contrast, killing of A375 cells by DNase fusions is negligible, consistent  
with negligible binding of the fusions to these cells.

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## Human DNase I

LOCUS HUMDNASEI 1039 bp mRNA PRI 06-MAR-1995  
 DEFINITION Human DNase I mRNA, complete cds.  
 ACCESSION M55983  
 VERSION M55983.1 GI:181623  
 KEYWORDS DNase I.  
 SOURCE Human pancreas, cDNA to mRNA.  
 ORGANISM Homo sapiens  
 Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Mammalia;  
 Eutheria; Primates; Catarrhini; Hominidae; Homo.  
 REFERENCE 1 (bases 1 to 1039)  
 AUTHORS Shak, S., Capon, D.J., Hellmiss, R., Marsters, S.A. and Baker, C.L.  
 TITLE Recombinant human DNase I reduces the viscosity of cystic fibrosis  
 sputum  
 JOURNAL Proc. Natl. Acad. Sci. U.S.A. 87 (23), 9188-9192 (1990)  
 MEDLINE 91067672  
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//

# Fig. 1

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## 2/113

### Human DNase I construct

LOCUS MHDNASE.DN 783 bp mRNA PRI 06-MAR-1995  
 DEFINITION Human DNase I mRNA, complete cds, Mature sequence modified to remove NarI site  
 ACCESSION M55983  
 NID g181623  
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 ORGANISM Homo sapiens  
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata; Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.  
 REFERENCE 1 (bases 1 to 1039)  
 AUTHORS Shak,S., Capon,D.J., Hellmiss,R., Marsters,S.A. and Baker,C.L.  
 TITLE Recombinant human DNase I reduces the viscosity of cystic fibrosis sputum  
 JOURNAL Proc. Natl. Acad. Sci. U.S.A. 87 (23), 9188-9192 (1990)  
 MEDLINE 91067672  
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601 GACACCACAG CTACACCCAC GCACTGTGCC TATGACAGGA TCGTGGTTGC AGGGATGCTG
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//

**Fig. 2(A)**

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ACCESSION -  
KEYWORDS -  
SOURCE -

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/clone="hDNase-18-1"  
/tissue\_type="pancreas [Split]"  
sig\_peptide 10..75  
/gene="DNase I"  
CDS 10..>75  
/gene="DNase I"  
/codon\_start="1"  
/product="DNase I"  
/db\_xref="PID:g181624"

/translation="MRGMKLLGALLALAALLQGAVS[LKIAAFNIQTFGETKMSNATLV

SYIVQILSRDYDIALVQEVDRSHLTAVGKLLDNLNQDAPDTYHYVVSEPLGRNSYKERY

LFVYRPDQVSAVDSYYYDDGCEPCGNDTFNREPAIVRFFSRFTEVREFAIVPLHAAPG

D... [Split]"  
gene 10..>75  
/gene="DNase I [Split]"  
frag 76..858  
/note="1 to 783 of mod humanDNaseI"  
frag 76..858  
/note="1 to 72 of 104linker"  
frag join(76..>129,<131..147)  
/note="1 to 72 of 103linker [Split]"  
frag join(76..>126,<127..>129,<131..147)  
/note="1 to 78 of 102linker [Split]"

BASE COUNT 177 A 260 C 251 G 170 T 0 OTHER

ORIGIN -

```

1  GCCGCCACCA TGAGGGGCAT GAAGCTGCTG GGGGCGCTGC TGGCACTGGC GGCCCTACTG
61 CAGGGGGGCG TGTCCTGAA GATCGCAGCC TTCAACATCC AGACATTTGG GGAGACCAAG
121 ATGTCCAATG CCACCCTCGT CAGCTACATT GTGCAGATCC TGAGCCGCTA CGACATCGCC
181 CTGGTCCAGG AGGTCAGAGA CAGCCACCTG ACTGCCGTGG GGAAGCTGCT GGACAACCTC
241 AATCAGGACG CACCAGACAC CTATCACTAC GTGGTCAGTG AGCCACTGGG ACGGAACAGC
301 TATAAGGAGC GCTACCTGTT CGTGTACAGG CCTGACCAGG TGTCTGCGGT GGACAGCTAC
361 TACTACGATG ATGGCTGCGA GCCCTGCGGG AACGACACCT TCAACCGAGA GCCAGCCATT
421 GTCAGGTTCT TCTCCCGGTT CACAGAGGTC AGGGAGTTTG CCATTGTTCC CCTGCATGCG
481 GCCCCGGGGG ACGCAGTAGC CGAGATCGAC GCTCTCTATG ACGTCTACCT GGATGTCCAA
541 GAGAAATGGG GCTTGGAGGA CGTCATGTTG ATGGGCGACT TCAATGCGGG CTGCAGCTAT
601 GTGAGACCTT CCCAGTGGTC ATCCATCCGC CTGTGGACAA GCCCCACCTT CCAGTGGCTG
661 ATCCCCGACA GCGCTGACAC CACAGCTACA CCCACGCACT GTGCCTATGA CAGGATCGTG
721 GTTGCAGGGA TGCTGCTCCG AGGGGCCGTT GTTCCCGACT CGGCTCTTCC CTTTAACTTC
781 CAGGCTGCCT ATGGCCTGAG TGACCAACTG GCCCAAGCCA TCAGTGACCA CTATCCAGTG
841 GAGGTGATGC TGAAGTGA

```

//

**Fig. 2(B)**

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**pAS6 – light chain**

LOCUS HMFG1LC2.D 721 bp DNA 18-AUG-1998  
 DEFINITION HUMANISED HMFG1 LIGHT CHAIN Vnp LEADER.  
 ACCESSION  
 KEYWORDS  
 SOURCE  
 ORGANISM  
 REFERENCE 1 (BASES 1 TO 342)  
 AUTHORS VERHOEYEN ET AL  
 TITLE CONSTRUCTION OF RESHAPED HMFG1 ETC  
 JOURNAL IMMUNOL. (1993):78, 364-370  
 COMMENT SCANNED IN FROM JOURNAL  
 FEATURES  
 SITES

This is the sequence of the HMFG1 light chain gene with the Vnp leader sequence attached. Translate from residue 1. Note residue 399 is T > A in all clones leading to R133 silent mutation (T in Verhoeven paper)

BASE COUNT 197 a 202 c 182 g 140 t  
 ORIGIN ?

```

x      LEADER SEQ
1  ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCGGAC
61  ATCCAGATGA CCCAGAGCCC AAGCAGCCTG AGCGCCAGCG TGGGTGACAG AGTGACCATC
121 ACCTGTAAGT CCAGTCAGAG CCTTTTATAT AGTAGCAATC AAAAGATCTA CTTGGCCTGG
181 TACCAGCAGA AGCCAGGTAA GGCTCCAAAG CTGCTGATCT ACTGGGCATC CACTAGGGAA
241 TCTGGTGTGC CAAGCAGATT CAGCGGTAGC GGTAGCGGTA CCGACTTCAC CTTCAACATC
301 AGCAGCCTCC AGCCAGAGGA CATCGCCACC TACTACTGCC AGCAATATTA TAGATATCCT
361 CGGACGTTTC GCCAAGGGAC CAAGGTGGAA ATCAAACGAA CTGTGGCTGC ACCATCTGTC
421 TTCATCTTCC CGCCATCTGA TGAGCAGTTG AAATCTGGAA CTGCCTCTGT TGTGTGCCTG
481 CTGAATAACT TCTATCCCAG AGAGGCCAAA GTACAGTGGA AGGTGGATAA CGCCCTCCAA
541 TCGGGTAACT CCCAGGAGAG TGTCACAGAG CAGGACAGCA AGGACAGCAC CTACAGCCTC
601 AGCAGCACCC TGACGCTGAG CAAAGCAGAC TACGAGAAAC ACAAAGTCTA CGCCTGCGAA
661 GTCACCCATC AGGGCCTGAG CTCGCCCGTC ACAAAGAGCT TCAACAGGGG AGAGTGTTAG
721 A

```

//

***Fig. 3(A)***

# 5/113

```

LOCUS      HHMFG1KLC_      730 BP SS-DNA      SYN      29-AUG-2000
DEFINITION -
ACCESSION  -
KEYWORDS   -
SOURCE     -
FEATURES   Location/Qualifiers
     frag   10..730
             /note="1 to 721 of hHMFG1light chain"
     frag   10..730
             /note="1 to 72 of 104linker"
     frag   join(10..>63,<65..81)
             /note="1 to 72 of 103linker [Split]"
     frag   join(10..>60,<61..>63,<65..81)
             /note="1 to 78 of 102linker [Split]"
BASE COUNT 198 A      208 C      184 G      140 T      0 OTHER
ORIGIN      -
      1  GCCGCCACCA TGGGATGGAG CTGTATCATC CTCTTCTTGG TAGCAACAGC TACAGGTGTC
     61  CACTCCGACA TCCAGATGAC CCAGAGCCCA AGCAGCCTGA GCGCCAGCGT GGGTGACAGA
    121  GTGACCATCA CCTGTAAGTC CAGTCAGAGC CTTTATATA GTAGCAATCA AAAGATCTAC
    181  TTGGCCTGGT ACCAGCAGAA GCCAGGTAAG GCTCCAAAGC TGCTGATCTA CTGGGCATCC
    241  ACTAGGGAAT CTGGTGTGCC AAGCAGATTC AGCGGTAGCG GTAGCGGTAC CGACTTCACC
    301  TTCACCATCA GCAGCCTCCA GCCAGAGGAC ATCGCCACCT ACTACTGCCA GCAATATTAT
    361  AGATATCCTC GGACGTTCCG CCAAGGGACC AAGGTGGAAA TCAAACGAAC TGTGGCTGCA
    421  CCATCTGTCT TCATCTTCCC GCCATCTGAT GAGCAGTTGA AATCTGGAAC TGCCTCTGTT
    481  GTGTGCCTGC TGAATAACTT CTATCCCAGA GAGGCCAAAG TACAGTGGAA GGTGGATAAC
    541  GCCCTCCAAT CGGGTAACTC CCAGGAGAGT GTCACAGAGC AGGACAGCAA GGACAGCACC
    601  TACAGCCTCA GCAGCACCTT GACGCTGAGC AAAGCAGACT ACGAGAAACA CAAAGTCTAC
    661  GCCTGCGAAG TCACCCATCA GGCCTGAGC TCGCCCGTCA CAAAGAGCTT CAACAGGGGA
    721  GAGTGTTAGA

```

//

***Fig. 3(B)***

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**HMFG-1 light chain with Vnp Leader (shaded)**

MGWSCIILFLVATATGVHSDIQMTQSPSSLSASVGDRVITITCKSSQSL  
LYSSNQKIYLAWYQQKPGKAPKLLIWASTRESGVPSRFSGSGSGT  
DFTFTISSLQPEDIATYYCQQYYRYPRTFGQGTKVEIKRTVAAPSVFI  
FPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESV  
TEQDSKDSSTLSSTLTLSKADYEKHKVYACEVTHQGLSPVTKSFN  
RGEC

***Fig. 3(C)***

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## pAS6 – heavy chain

LOCUS HHMFG1HC.D 1404 bp DNA 14-AUG-1998  
 DEFINITION HUMANISED HMFG1 heavy chain  
 ACCESSION HHMFG1H  
 KEYWORDS  
 SOURCE  
 ORGANISM  
 REFERENCE  
 AUTHORS VERHOEYEN ET AL  
 TITLE CONSTRUCTION OF RESHAPED HMFG1 etc  
 JOURNAL IMMUNOL. (1993):78, 364-370  
 COMMENT VH domain SCANNED IN FROM JOURNAL  
 FEATURES AA RESIDUE 235 HAS NOT BEEN CHANGED TO KABAT (I.E. V TO A)  
 FEATURES Residue 963 is G > T leading to silent mutation in all clones  
 SITES Note  
 BASE COUNT 333 a 439 c 379 g 253 t  
 ORIGIN ?

← LEADER

```

1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCAG
61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
481 GCCCTGGGCT GCCTGGTCAA GGACTIONTC CCCGAACCGG TGACGGTGTC GTGGAACCTCA
541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
661 AACGTGAATC ACAAGCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
721 GACAAAATCT ACACATGCCC ACCGTGCCCC GCACCTGAAC TCCTGGGGGG ACCGTCAATC
781 TTCCTCTTCC CCCCAAAACC CAAGGACACC CTCATGATCT CCCGGACCCC TGAGGTACAC
841 TGGGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC
901 GGCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC
961 CGTGTGGTCA GCTCCTCAC CGTCTGCAC CAGGACTGGC TGAATGGCAA GGAGTACAAG
1021 TGCAAGGTCT CCAACAAAGC CCTCCAGGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA
1081 GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCCGGGATGA GCTGACCAAG
1141 AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG
1201 TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT GCTGGACTCC
1261 GACGGCTCCT TCTTCTCTA CAGCAAGCTC ACCGTGGACA AGAGCAGGTG GCAGCAGGGG
1321 AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC
1381 CTCTCCCTGT CTCCGGGTAA ATGA
  
```

Antibody DNase Fusions Made Here  
(eg pAS34----39.)

End of lower hinge region of heavy chain. PAPE Amino  
Acid Seq. Fab'<sub>2</sub> fusions were made at this point.

Those with HYBRID HINGES are altered further  
up i.e.

This part GACAAAATGACACA  
D K T H T

After this sequence you get the HYBRID HINGE + LINKER SEQUENCES  
Then DNase I (eg Fab-DNase construct pAS302)

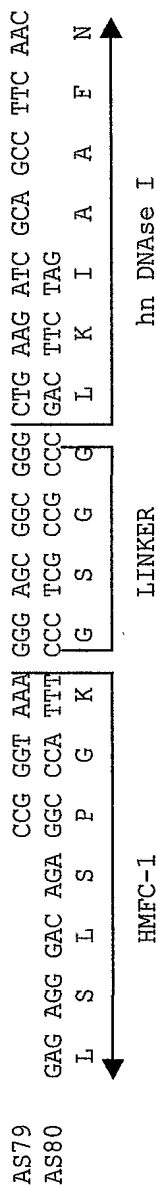
**Fig. 3(D)**

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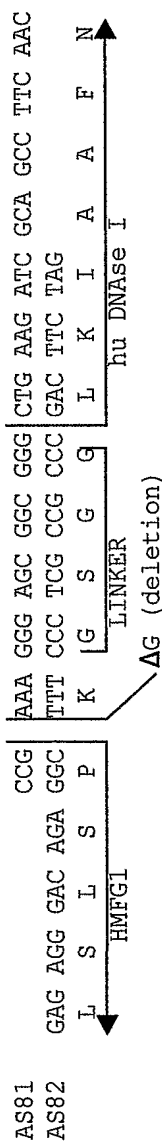
**Fig. 4(A)**

Oligos involved in the fusion of whole antibody-DNase

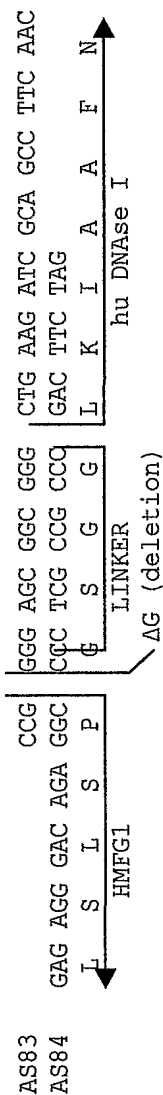
Constructs pAS34/37



Constructs pAS35/38

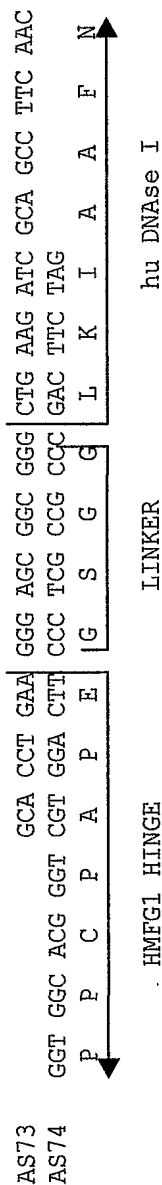


Constructs pAS36/39



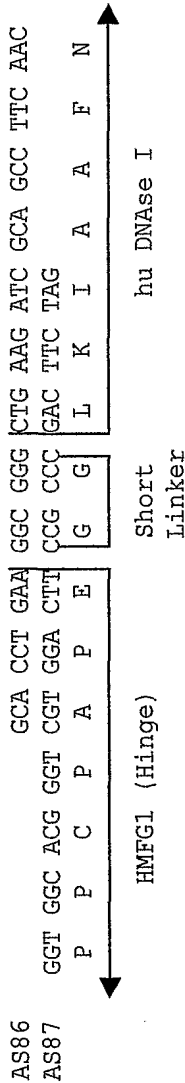
Oligos involved in the fusion of Fab'2-DNaseI

Constructs pAS23/27

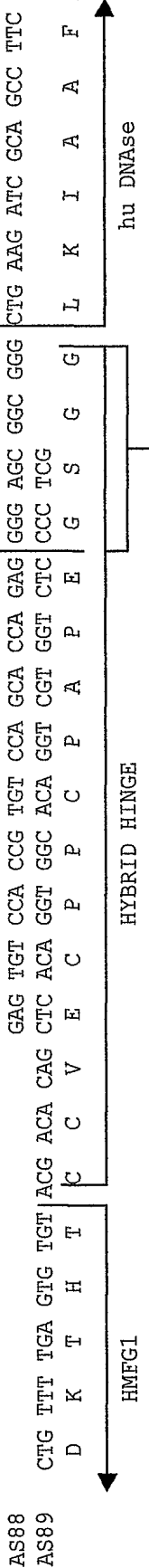


Oligos involved in the fusion of new Fab'2-DNaseI molecules (5.7.99)

Constructs pAS101/105



Constructs pAS102/106



Constructs pAS103/107

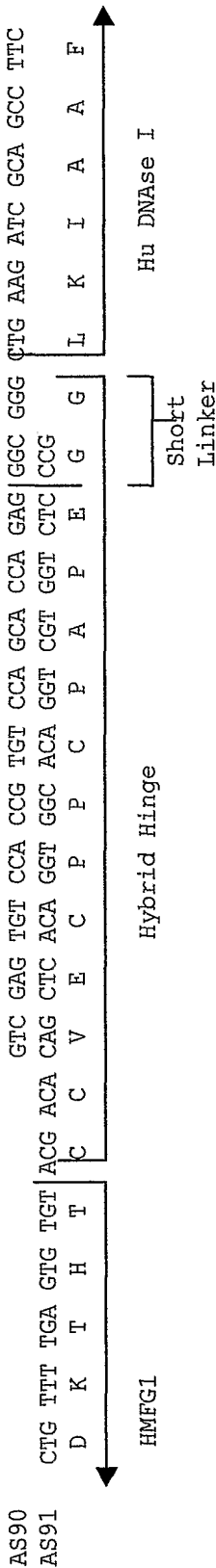


Fig. 4(B)

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pAS23

LOCUS PAS23.DNA 1554 bp mRNA PRI 06-MAR-1995  
 DEFINITION Humanised HMFG1 Fab'2 fused to human DNase I (construct 1)  
 ACCESSION  
 NID  
 KEYWORDS DNase I.  
 SOURCE DNase I sequence is from assembled oligos (thus modified c/f  
 MHDNASE1.dna)  
 ORGANISM Homo sapiens  
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;  
 Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.  
 AUTHORS Shak,S., Capon,D.J., Hellmiss,R., Marsters,S.A. and Baker,C.L.  
 TITLE Recombinant human DNase I reduces the viscosity of cystic  
 fibrosis  
 sputum  
 JOURNAL Proc. Natl. Acad. Sci. U.S.A. 87 (23), 9188-9192 (1990)  
 MEDLINE 91067672  
 BASE COUNT 344 a 468 c 434 g 308 t  
 ORIGIN

```

1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
121 TGCAAGGCTT CTGGCTACAC CTTCAAGTGC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAAGTCA
541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
721 GACAAAATC ACACATGCCC ACCGTGCCCC GCACCTGAAG GGAGCGGCGG GCTGAAGATC
781 GCAGCCTTCA ACATCCAGAC ATTTGGGGAG ACCAAGATGT CCAATGCCAC CCTCGTCAGC
841 TACATTGTGC AGATCCTGAG CCGCTACGAC ATCGCCCTGG TCCAGGAGGT CAGAGACAGC
901 CACCTGACTG CCGTGGGGAA GCTGCTGGAC AACCTCAATC AGGACGCACC AGACACCTAT
961 CACTACGTGG TCAGTGAGCC ACTGGGACGG AACAGCTATA AGGAGCGCTA CCTGTTCTGT
1021 TACAGGCCTG ACCAGGTGTC TGCGGTGGAC AGCTACTACT ACGATGATGG CTGCGAGCCC
1081 TGCGGGAACG ACACCTTCAA CCGAGAGCCA GCCATTGTCA GGTCTTCTC CCGGTTTACA
1141 GAGGTCAGGG AGTTTGCCAT TGTTCCCCTG CATGCGGCCC CGGGGGACGC AGTAGCCGAG
1201 ATCGACGCTC TCTATGACGT CTACCTGGAT GTCCAAGAGA AATGGGGCTT GGAGGACGTC
1261 ATGTTGATGG GCGACTTCAA TGCGGGCTGC AGCTATGTGA GACCCTCCCA GTGGTCATCC
1321 ATCCGCCTGT GGACAAGCCC CACCTTCCAG TGGCTGATCC CCGACAGCGC TGACACCACA
1381 GCTACACCCA CGCACTGTGC CTATGACAGG ATCGTGTTG CAGGGATGCT GCTCCGAGGG
1441 GCCGTTGTTC CCGACTCGGC TCTTCCCTTT AACTTCCAGG CTGCCTATGG CCTGAGTGAC
1501 CAACTGGCCC AAGCCATCAG TGACCACTAT CCAGTGAGAG TGATGCTGAA GTGA

```

//

*Fig. 5(A)*

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LOCUS FDDNASE23\_ 1554 BP SS-DNA SYN 25-AUG-2000  
 DEFINITION -  
 ACCESSION -  
 KEYWORDS -  
 SOURCE -  
 FEATURES Location/Qualifiers  
     frag join(1..>720,<787..1554)  
           /note="1 to 1554 of 23.dna [Split]"  
     frag 721..786  
           /note="1 to 66 of 23/27linker"  
     frag join(721..>735,<736..786)  
           /note="1 to 78 of 102linker [Split]"  
 BASE COUNT 344 A 466 C 435 G 309 T 0 OTHER  
 ORIGIN -  
 1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG  
 61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC  
 121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA  
 181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT  
 241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG  
 301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC  
 361 TTTGCCCTGG TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC  
 421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG  
 481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA  
 541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC  
 601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC  
 661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT  
 721 GACAAAAC TC ACATGTCC ACCGTGTCCA GCACCAGAGG GGAGCGGCGG GCTGAAGATC  
 781 GCAGCCTTCA ACATCCAGAC ATTTGGGGAG ACCAAGATGT CCAATGCCAC CCTCGTCAGC  
 841 TACATTGTGC AGATCCTGAG CCGTACGAC ATCGCCCTGG TCCAGGAGGT CAGAGACAGC  
 901 CACCTGACTG CCGTGGGGAA GCTGCTGGAC AACCTCAATC AGGACGCACC AGACACCTAT  
 961 CACTACGTGG TCAGTGAGCC ACTGGGACGG AACAGCTATA AGGAGCGCTA CCGTTCGTG  
 1021 TACAGGCCTG ACCAGGTGTC TCGGGTGGAC AGCTACTACT ACGATGATGG CTGCGAGCCC  
 1081 TGCGGGAACG ACACCTTCAA CCGAGAGCCA GCCATTGTCA GGTTCTTCTC CCGGTTTACA  
 1141 GAGGTCAGGG AGTTTGCCAT TGTTCCCCTG CATGCGGCCC CGGGGGACGC AGTAGCCGAG  
 1201 ATCGACGCTC TCTATGACGT CTACCTGGAT GTCCAAGAGA AATGGGGCTT GGAGGACGTC  
 1261 ATGTTGATGG GCGACTTCAA TGCGGGCTGC AGCTATGTGA GACCCTCCCA GTGGTCATCC  
 1321 ATCCGCCTGT GGACAAGCCC CACCTTCCAG TGGCTGATCC CCGACAGCGC TGACACCACA  
 1381 GCTACACCCA CGCACTGTGC CTATGACAGG ATCGTGGTTG CAGGGATGCT GCTCCGAGGG  
 1441 GCCGTTGTTT CCGACTCGGC TCTTCCCTTT AACTTCCAGG CTGCCTATGG CCTGAGTGAC  
 1501 CAACTGGCCC AAGCCATCAG TGACCACTAT CCACTGGAGG TGATGCTGAA GTGA

//

*Fig. 5(B)*

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LOCUS FDDNASE23K 1563 BP SS-DNA SYN 29-AUG-2000  
 DEFINITION -  
 ACCESSION -  
 KEYWORDS -  
 SOURCE -  
 FEATURES Location/Qualifiers  
     frag 10..1563  
         /note="1 to 1554 of FdDNase23correct"  
     frag join(10..>729,<796..1563)  
         /note="1 to 1554 of 23.dna [Split]"  
     frag 730..795  
         /note="1 to 66 of 23/27linker"  
     frag join(730..>744,<745..795)  
         /note="1 to 78 of 102linker [Split]"  
 BASE COUNT 345 A 472 C 437 G 309 T 0 OTHER  
 ORIGIN -  
     1 GCCGCCACCA TGGGATGGAG CTGTATCATC CTCTTCTTGG TAGCAACAGC TACAGGTGTC  
     61 CACTCCCAGG TGCAGCTGGT GCAGTCTGGG GCAGAGGTGA AAAAGCCTGG GGCCCTCAGTG  
     121 AAGGTGTCCT GCAAGGCTTC TGGCTACACC TTCAGTGCCT ACTGGATAGA GTGGGTGCGC  
     181 CAGGCTCCAG GAAAGGGCCT CGAGTGGGTC GGAGAGATTT TACCTGGAAG TAATAATTCT  
     241 AGATACAATG AGAAGTTCAA GGGCCGAGTG ACAGTCACTA GAGACACATC CACAAACACA  
     301 GCCTACATGG AGCTCAGCAG CCTGAGGTCT GAGGACACAG CCGTCTATTA CTGTGCAAGA  
     361 TCCTACGACT TTGCCTGGTT TGCTTACTGG GGCCAAGGGA CTCTGGTCAC AGTCTCCTCA  
     421 GCCTCCACCA AGGGCCCATC GGTCTTCCCC CTGGCACCCCT CCTCCAAGAG CACCTCTGGG  
     481 GGCACAGCGG CCCTGGGCTG CCTGGTCAAG GACTACTTCC CCGAACCGGT GACGGTGTCTG  
     541 TGGAACTCAG GCGCCCTGAC CAGCGGCGTG CACACCTTCC CGGCTGTCCCT ACAGTCCTCA  
     601 GGA CTCTACT CCCTCAGCAG CGTGGTGACC GTGCCCTCCA GCAGCTTGGG CACCCAGACC  
     661 TACATCTGCA ACGTGAATCA CAAGCCCAGC AACACCAAGG TGGACAAGAA AGTTGAGCCC  
     721 AAATCTTGTG ACAA AACTCA CACATGTCCA CCGTGTCCAG CACCAGAGGG GAGCGGCGGG  
     781 CTGAAGATCG CAGCCTTCAA CATCCAGACA TTTGGGGAGA CCAAGATGTC CAATGCCACC  
     841 CTCGTCAGCT ACATTGTGCA GATCCTGAGC CGCTACGACA TCGCCCTGGT CCAGGAGGTC  
     901 AGAGACAGCC ACCTGACTGC CGTGGGGAAG CTGCTGGACA ACCTCAATCA GGACGCACCA  
     961 GACACCTATC ACTACGTGGT CAGTGAGCCA CTGGGACGGA ACAGCTATAA GGAGCGCTAC  
     1021 CTGTTTCGTGT ACAGGCCTGA CCAGGTGTCT GCGGTGGACA GCTACTACTA CGATGATGGC  
     1081 TGCGAGCCCT GCGGGAACGA CACCTTCAAC CGAGAGCCAG CCATTGTCAG GTTCTTCTCC  
     1141 CGGTTACAG AGGTCAGGGA GTTTGCCATT GTTCCCCTGC ATGCGGCCCC GGGGGACGCA  
     1201 GTAGCCGAGA TCGACGCTCT CTATGACGTC TACCTGGATG TCCAAGAGAA ATGGGGCTTG  
     1261 GAGGACGTCA TGTTGATGGG CGACTTCAAT GCGGGCTGCA GCTATGTGAG ACCCTCCCAG  
     1321 TGGTCATCCA TCCGCCTGTG GACAAGCCCC ACCTTCCAGT GGCTGATCCC CGACAGCGCT  
     1381 GACACCACAG CTACACCCAC GCACTGTGCC TATGACAGGA TCGTGGTTGC AGGGATGCTG  
     1441 CTCCGAGGGG CCGTTGTTCC CGACTCGGCT CTTCCCTTTA ACTTCCAGGC TGCCTATGGC  
     1501 CTGAGTGACC AACTGGCCCA AGCCATCAGT GACCACTATC CAGTGGAGGT GATGCTGAAG  
     1561 TGA

//

*Fig. 5(C)*

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		9			18			27			36			45			54	
5'	ATG	GGA	TGG	AGC	TGT	ATC	ATC	CTC	TTC	TTG	GTA	GCA	ACA	GCT	ACA	GGT	GTC	CAC
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	M	G	W	S	C	I	I	L	F	L	V	A	T	A	T	G	V	H
			63			72			81			90			99			108
	TCC	CAG	GTG	CAG	CTG	GTG	CAG	TCT	GGG	GCA	GAG	GTG	AAA	AAG	CCT	GGG	GCC	TCA
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	S	Q	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S
			117			126			135			144			153			162
	GTG	AAG	GTG	TCC	TGC	AAG	GCT	TCT	GGC	TAC	ACC	TTC	AGT	GCC	TAC	TGG	ATA	GAG
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	V	K	V	S	C	K	A	S	G	Y	T	F	S	A	Y	W	I	E
			171			180			189			198			207			216
	TGG	GTG	CGC	CAG	GCT	CCA	GGA	AAG	GGC	CTC	GAG	TGG	GTC	GGA	GAG	ATT	TTA	CCT
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	W	V	R	Q	A	P	G	K	G	L	E	W	V	G	E	I	L	P
			225			234			243			252			261			270
	GGA	AGT	AAT	AAT	TCT	AGA	TAC	AAT	GAG	AAG	TTC	AAG	GGC	CGA	GTG	ACA	GTC	ACT
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	G	S	N	N	S	R	Y	N	E	K	F	K	G	R	V	T	V	T
			279			288			297			306			315			324
	AGA	GAC	ACA	TCC	ACA	AAC	ACA	GCC	TAC	ATG	GAG	CTC	AGC	AGC	CTG	AGG	TCT	GAG
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	R	D	T	S	T	N	T	A	Y	M	E	L	S	S	L	R	S	E
			333			342			351			360			369			378
	GAC	ACA	GCC	GTC	TAT	TAC	TGT	GCA	AGA	TCC	TAC	GAC	TTT	GCC	TGG	TTT	GCT	TAC
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	D	T	A	V	Y	Y	C	A	R	S	Y	D	F	A	W	F	A	Y
			387			396			405			414			423			432
	TGG	GGC	CAA	GGG	ACT	CTG	GTC	ACA	GTC	TCC	TCA	GCC	TCC	ACC	AAG	GGC	CCA	TCG
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	W	G	Q	G	T	L	V	T	V	S	S	A	S	T	K	G	P	S
			441			450			459			468			477			486
	GTC	TTC	CCC	CTG	GCA	CCC	TCC	TCC	AAG	AGC	ACC	TCT	GGG	GGC	ACA	GCG	GCC	CTG
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	V	F	P	L	A	P	S	S	K	S	T	S	G	G	T	A	A	L
			495			504			513			522			531			540
	GGC	TGC	CTG	GTC	AAG	GAC	TAC	TTC	CCC	GAA	CCG	GTG	ACG	GTG	TCG	TGG	AAC	TCA
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	G	C	L	V	K	D	Y	F	P	E	P	V	T	V	S	W	N	S
			549			558			567			576			585			594
	GGC	GCC	CTG	ACC	AGC	GGC	GTG	CAC	ACC	TTC	CCG	GCT	GTC	CTA	CAG	TCC	TCA	GGA
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	G	A	L	T	S	G	V	H	T	F	P	A	V	L	Q	S	S	G

**Fig. 5(D)**  
(Sheet 1 of 3)

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603	612	621	630	639	648
CTC TAC TCC CTC AGC	AGC GTG GTG ACC GTG CCC	TCC AGC AGC TTG GGC	ACC CAG		
---	---	---	---	---	---
L Y S L S S V V T V P S S S L G T Q					
657	666	675	684	693	702
ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA					
---	---	---	---	---	---
T Y I C N V N H K P S N T K V D K K					
711	720	729	738	747	756
GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT					
---	---	---	---	---	---
V E P K S C D K T H T C P P C P A P					
765	774	783	792	801	810
GAA GGG AGC GGC GGG CTG AAG ATC GCA GCC TTC AAC ATC CAG ACA TTT GGG GAG					
---	---	---	---	---	---
E G S G G L K I A A F N I Q T F G E					
819	828	837	846	855	864
ACC AAG ATG TCC AAT GCC ACC CTC GTC AGC TAC ATT GTG CAG ATC CTG AGC CGC					
---	---	---	---	---	---
T K M S N A T L V S Y I V Q I L S R					
873	882	891	900	909	918
TAC GAC ATC GCC CTG GTC CAG GAG GTC AGA GAC AGC CAC CTG ACT GCC GTG GGG					
---	---	---	---	---	---
Y D I A L V Q E V R D S H L T A V G					
927	936	945	954	963	972
AAG CTG CTG GAC AAC CTC AAT CAG GAC GCA CCA GAC ACC TAT CAC TAC GTG GTC					
---	---	---	---	---	---
K L L D N L N Q D A P D T Y H Y V V					
981	990	999	1008	1017	1026
AGT GAG CCA CTG GGA CGG AAC AGC TAT AAG GAG CGC TAC CTG TTC GTG TAC AGG					
---	---	---	---	---	---
S E P L G R N S Y K E R Y L F V Y R					
1035	1044	1053	1062	1071	1080
CCT GAC CAG GTG TCT GCG GTG GAC AGC TAC TAC TAC GAT GAT GGC TGC GAG CCC					
---	---	---	---	---	---
P D Q V S A V D S Y Y Y D D G C E P					
1089	1098	1107	1116	1125	1134
TGC GGG AAC GAC ACC TTC AAC CGA GAG CCA GCC ATT GTC AGG TTC TTC TCC CGG					
---	---	---	---	---	---
C G N D T F N R E P A I V R F F S R					
1143	1152	1161	1170	1179	1188
TTC ACA GAG GTC AGG GAG TTT GCC ATT GTT CCC CTG CAT GCG GCC CCG GGG GAC					
---	---	---	---	---	---
F T E V R E F A I V P L H A A P G D					
1197	1206	1215	1224	1233	1242

**Fig. 5(D)**  
(Sheet 2 of 3)

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```

GCA GTA GCC GAG ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT GTC CAA GAG AAA
-----
A   V   A   E   I   D   A   L   Y   D   V   Y   L   D   V   Q   E   K

      1251      1260      1269      1278      1287      1296
TGG GGC TTG GAG GAC GTC ATG TTG ATG GGC GAC TTC AAT GCG GGC TGC AGC TAT
-----
W   G   L   E   D   V   M   L   M   G   D   F   N   A   G   C   S   Y

      1305      1314      1323      1332      1341      1350
GTG AGA CCC TCC CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC CCC ACC TTC CAG
-----
V   R   P   S   Q   W   S   S   I   R   L   W   T   S   P   T   F   Q

      1359      1368      1377      1386      1395      1404
TGG CTG ATC CCC GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG CAC TGT GCC TAT
-----
W   L   I   P   D   S   A   D   T   T   A   T   P   T   H   C   A   Y

      1413      1422      1431      1440      1449      1458
GAC AGG ATC GTG GTT GCA GGG ATG CTG CTC CGA GGG GCC GTT GTT CCC GAC TCG
-----
D   R   I   V   V   A   G   M   L   L   R   G   A   V   V   P   D   S

      1467      1476      1485      1494      1503      1512
GCT CTT CCC TTT AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC CAA CTG GCC CAA
-----
A   L   P   F   N   F   Q   A   A   Y   G   L   S   D   Q   L   A   Q

      1521      1530      1539      1548
GCC ATC AGT GAC CAC TAT CCA GTG GAG GTG ATG CTG AAG TGA 3'
-----
A   I   S   D   H   Y   P   V   E   V   M   L   K   *

```

***Fig. 5(D)***  
***(Sheet 3 of 3)***

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pAS27

LOCUS PAS27.DNA 1584 bp mRNA PRI 06-MAR-1995  
 DEFINITION Humanised HMFG1 Fab'2 fused to human DNase I with SV40  
 NLS(construct 1)  
 ACCESSION  
 NID  
 KEYWORDS DNase I.  
 SOURCE DNase I sequence is from assembled oligos (thus modified c/f  
 MHDNASE1.dna)  
 ORGANISM Homo sapiens  
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;  
 Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.  
 AUTHORS Shak, S., Capon, D.J., Hellmiss, R., Marsters, S.A. and Baker, C.L.  
 TITLE Recombinant human DNase I reduces the viscosity of cystic fibrosis  
 sputum  
 JOURNAL Proc. Natl. Acad. Sci. U.S.A. 87 (23), 9188-9192 (1990)  
 MEDLINE 91067672  
 BASE COUNT 354 a 474 c 446 g 310 t  
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1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
61 GTGCAGCTGG TGCACTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
121 TGCAAGGCTT CTGGCTACAC CTTCACTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
481 GCCCTGGGCT GCCTGGTCAA GGAATACTTC CCCGAACCGG TGACGGTGTG GTGGAAGTCA
541 GCGGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
721 GACAAAACCT ACACATGCCC ACCGTGCCCC GCACCTGAAG GGAGCGGCGG GCTGAAGATC
781 GCAGCCTTCA ACATCCAGAC ATTTGGGGAG ACCAAGATGT CCAATGCCAC CCTCGTCAGC
841 TACATTGTGC AGATCCTGAG CCGCTACGAC ATCGCCCTGG TCCAGGAGGT CAGAGACAGC
901 CACCTGACTG CCGTGGGGAA GCTGCTGGAC AACCTCAATC AGGACGCACC AGACACCTAT
961 CACTACGTGG TCAGTGAGCC ACTGGGACGG AACAGCTATA AGGAGCGCTA CCTGTTCTGT
1021 TACAGGCCCT ACCAGGTGTC TGCGGTGGAC AGCTACTACT ACGATGATGG CTGCGAGCCC
1081 TGCGGGAACG ACACCTTCAA CCGAGAGCCA GCCATTGTCA GGTTCTTCTC CCGGTTTACA
1141 GAGGTCAGGG AGTTTGCCAT TGTTCCCTTG CATGCGGCCC CGGGGGACGC AGTAGCCGAG
1201 ATCGACGCTC TCTATGACGT CTACCTGGAT GTCCAAGAGA AATGGGGCTT GGAGGACGTC
1261 ATGTTGATGG GCGACTTCAA TGCGGGCTGC AGCTATGTGA GACCCTCCCA GTGGTCATCC
1321 ATCCGCCTGT GGACAAGCCC CACCTTCCAG TGGCTGATCC CCGACAGCGC TGACACCACA
1381 GCTACACCCA CGCACTGTGC CTATGACAGG ATCGTGGTTG CAGGGATGCT GCTCCGAGGG
1441 GCCGTTGTTT CCGACTCGGC TCTTCCCTTT AACTTCCAGG CTGCCTATGG CCTGAGTGAC
1501 CAACGGGCCC AAGCCATCAG TGACCACTAT CCAGTGGAGG TGATGCTGAA GGGGGGCGGA
1561 CCCAAAAAGA AGCGCAAGGT TTGA

```

//

*Fig. 6(A)*

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 ACCESSION -  
 KEYWORDS -  
 SOURCE -  
 FEATURES Location/Qualifiers  
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    61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC  
 121 TGCAAGGCTT CTGGCTACAC CTTCACTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA  
 181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT  
 241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG  
 301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC  
 361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC  
 421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG  
 481 GCCCTGGGCT GCCTGGTCAA GGACTIONTC CCCGAACCGG TGACGGTGTC GTGGAACCTCA  
 541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC  
 601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC  
 661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT  
 721 GACAAAACCTC ACACATGTCC ACCGTGTCCA GCACCAGAGG GGAGCGGCGG GCTGAAGATC  
 781 GCAGCCTTCA ACATCCAGAC ATTTGGGGGAG ACCAAGATGT CCAATGCCAC CCTCGTCAGC  
 841 TACATTGTGC AGATCCTGAG CCGTACGAC ATCGCCCTGG TCCAGGAGGT CAGAGACAGC  
 901 CACCTGACTG CCGTGGGGAA GCTGCTGGAC AACCTCAATC AGGACGCACC AGACACCTAT  
 961 CACTACGTGG TCAGTGAGCC ACTGGGACGG AACAGCTATA AGGAGCGCTA CCTGTTCGTG  
 1021 TACAGGCCTG ACCAGGTGTC TGCGGTGGAC AGCTACTACT ACGATGATGG CTGCGAGCCC  
 1081 TGCGGGAACG ACACCTTCAA CCGAGAGCCA GCCATTGTCA GGTTCCTCTC CCGGTTTACA  
 1141 GAGGTCAGGG AGTTTGCCAT TGTTCCTCTG CATGCGGCCC CGGGGGACGC AGTAGCCGAG  
 1201 ATCGACGCTC TCTATGACGT CTACCTGGAT GTCCAAGAGA AATGGGGCTT GGAGGACGTC  
 1261 ATGTTGATGG GCGACTTCAA TGCGGGCTGC AGCTATGTGA GACCCTCCCA GTGGTCATCC  
 1321 ATCCGCCTGT GGACAAGCCC CACCTTCCAG TGGCTGATCC CCGACAGCGC TGACACCACA  
 1381 GCTACACCCA CGCACTGTGC CTATGACAGG ATCGTGGTTG CAGGGATGCT GCTCCGAGGG  
 1441 GCCGTTGTTT CCGACTCGGC TCTTCCCTTT AACTTCCAGG CTGCCTATGG CCTGAGTGAC  
 1501 CAACTGGCCC AAGCCATCAG TGACCACTAT CCAGTGGAGG TGATGCTGAA GGGGGGCGGA  
 1561 CCCAAAAAGA AGCGCAAGGT TTGA

//

*Fig. 6(B)*

## 18/113

LOCUS FDDNASE27K 1593 BP SS-DNA SYN 29-AUG-2000  
 DEFINITION -  
 ACCESSION -  
 KEYWORDS -  
 SOURCE -  
 FEATURES Location/Qualifiers  
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     /note="1 to 78 of 102linker [Split]"  
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 ORIGIN -  
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  61 CACTCCCAGG TGCAGCTGGT GCAGTCTGGG GCAGAGGTGA AAAAGCCTGG GGCCTCAGTG  
 121 AAGGTGTCCT GCAAGGCTTC TGGCTACACC TTCAGTGCCT ACTGGATAGA GTGGGTGCGC  
 181 CAGGCTCCAG GAAAGGGCCT CGAGTGGGTC GGAGAGATTT TACCTGGAAG TAATAATTCT  
 241 AGATACAATG AGAAGTTCAA GGGCCGAGTG ACAGTCACTA GAGACACATC CACAAACACA  
 301 GCCTACATGG AGCTCAGCAG CCTGAGGTCT GAGGACACAG CCGTCTATTA CTGTGCAAGA  
 361 TCCTACGACT TTGCCTGGTT TGCTTACTGG GGCCAAGGGA CTCTGGTCAC AGTCTCCTCA  
 421 GCCTCCACCA AGGGCCCATC GGTCTTCCCC CTGGCACCCCT CCTCCAAGAG CACCTCTGGG  
 481 GGCACAGCGG CCCTGGGCTG CCTGGTCAAG GACTACTTCC CCGAACCGGT GACGGTGTCG  
 541 TGGAACCTCAG GCGCCCTGAC CAGCGGCGTG CACACCTTCC CGGCTGTCTT ACAGTCCTCA  
 601 GGA CTCTACT CCCTCAGCAG CGTGGTGACC GTGCCCTCCA GCAGCTTGGG CACCCAGACC  
 661 TACATCTGCA ACGTGAATCA CAAGCCCAGC AACACCAAGG TGGACAAGAA AGTTGAGCCC  
 721 AAATCTTGTG ACAAAACTCA CACATGTCCA CCGTGTCCAG CACCAGAGGG GAGCGGCGGG  
 781 CTGAAGATCG CAGCCTTCAA CATCCAGACA TTTGGGGAGA CCAAGATGTC CAATGCCACC  
 841 CTCGTCAGCT ACATTGTGCA GATCCTGAGC CGCTACGACA TCGCCCTGGT CCAGGAGGTC  
 901 AGAGACAGCC ACCTGACTGC CGTGGGGAAG CTGCTGGACA ACCTCAATCA GGACGCACCA  
 961 GACACCTATC ACTACGTGGT CAGTGAGCCA CTGGGACGGA ACAGCTATAA GGAGCGCTAC  
 1021 CTGTTTCGTGT ACAGGCCTGA CCAGGTGTCT GCGGTGGACA GCTACTACTA CGATGATGGC  
 1081 TGCGAGCCCT GCGGGAACGA CACCTTCAAC CGAGAGCCAG CCATTGTCAG GTTCTTCTCC  
 1141 CGGTTACAG AGGTCAGGGA GTTTGCCATT GTTCCCCTGC ATGCGGCCCC GGGGGACGCA  
 1201 GTAGCCGAGA TCGACGCTCT CTATGACGTC TACCTGGATG TCCAAGAGAA ATGGGGCTTG  
 1261 GAGGACGTCA TGTTGATGGG CGACTTCAAT GCGGGCTGCA GCTATGTGAG ACCCTCCAG  
 1321 TGGTCATCCA TCCGCCTGTG GACAAGCCCC ACCTTCCAGT GGCTGATCCC CGACAGCGCT  
 1381 GACACCACAG CTACACCCAC GCACTGTGCC TATGACAGGA TCGTGGTTGC AGGGATGCTG  
 1441 CTCCGAGGGG CCGTTGTTCC CGACTCGGCT CTTCCCTTTA ACTTCCAGGC TGCCTATGGC  
 1501 CTGAGTGACC AACTGGCCCA AGCCATCAGT GACCACTATC CAGTGAGGTT GATGCTGAAG  
 1561 GGGGGCGGAC CCAAAAAGAA GCGCAAGGTT TGA

//

*Fig. 6(C)*

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```

      9      18      27      36      45      54
5'  ATG GGA TGG AGC TGT ATC ATC CTC TTC TTG GTA GCA ACA GCT ACA GGT GTC CAC
    ---
    M  G  W  S  C  I  I  L  F  L  V  A  T  A  T  G  V  H

      63      72      81      90      99      108
TCC CAG GTG CAG CTG GTG CAG TCT GGG GCA GAG GTG AAA AAG CCT GGG GCC TCA
    ---
    S  Q  V  Q  L  V  Q  S  G  A  E  V  K  K  P  G  A  S

      117      126      135      144      153      162
GTG AAG GTG TCC TGC AAG GCT TCT GGC TAC ACC TTC AGT GCC TAC TGG ATA GAG
    ---
    V  K  V  S  C  K  A  S  G  Y  T  F  S  A  Y  W  I  E

      171      180      189      198      207      216
TGG GTG CGC CAG GCT CCA GGA AAG GGC CTC GAG TGG GTC GGA GAG ATT TTA CCT
    ---
    W  V  R  Q  A  P  G  K  G  L  E  W  V  G  E  I  L  P

      225      234      243      252      261      270
GGA AGT AAT AAT TCT AGA TAC AAT GAG AAG TTC AAG GGC CGA GTG ACA GTC ACT
    ---
    G  S  N  N  S  R  Y  N  E  K  F  K  G  R  V  T  V  T

      279      288      297      306      315      324
AGA GAC ACA TCC ACA AAC ACA GCC TAC ATG GAG CTC AGC AGC CTG AGG TCT GAG
    ---
    R  D  T  S  T  N  T  A  Y  M  E  L  S  S  L  R  S  E

      333      342      351      360      369      378
GAC ACA GCC GTC TAT TAC TGT GCA AGA TCC TAC GAC TTT GCC TGG TTT GCT TAC
    ---
    D  T  A  V  Y  Y  C  A  R  S  Y  D  F  A  W  F  A  Y

      387      396      405      414      423      432
TGG GGC CAA GGG ACT CTG GTC ACA GTC TCC TCA GCC TCC ACC AAG GGC CCA TCG
    ---
    W  G  Q  G  T  L  V  T  V  S  S  A  S  T  K  G  P  S

      441      450      459      468      477      486
GTC TTC CCC CTG GCA CCC TCC TCC AAG AGC ACC TCT GGG GGC ACA GCG GCC CTG
    ---
    V  F  P  L  A  P  S  S  K  S  T  S  G  G  T  A  A  L

      495      504      513      522      531      540
GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG AAC TCA
    ---
    G  C  L  V  K  D  Y  F  P  E  P  V  T  V  S  W  N  S

      549      558      567      576      585      594
GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA
    ---
    G  A  L  T  S  G  V  H  T  F  P  A  V  L  Q  S  S  G

```

**Fig. 6(D)**  
(Sheet 1 of 3)

## 20/113

603	612	621	630	639	648
CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACC CAG					
---	---	---	---	---	---
L Y S L S S V V T V P S S S L G T Q					
657	666	675	684	693	702
ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA					
---	---	---	---	---	---
T Y I C N V N H K P S N T K V D K K					
711	720	729	738	747	756
GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT					
---	---	---	---	---	---
V E P K S C D K T H T C P P C P A P					
765	774	783	792	801	810
GAA GGG AGC GGC GGG CTG AAG ATC GCA GCC TTC AAC ATC CAG ACA TTT GGG GAG					
---	---	---	---	---	---
E G S G G L K I A A F N I Q T F G E					
819	828	837	846	855	864
ACC AAG ATG TCC AAT GCC ACC CTC GTC AGC TAC ATT GTG CAG ATC CTG AGC CGC					
---	---	---	---	---	---
T K M S N A T L V S Y I V Q I L S R					
873	882	891	900	909	918
TAC GAC ATC GCC CTG GTC CAG GAG GTC AGA GAC AGC CAC CTG ACT GCC GTG GGG					
---	---	---	---	---	---
Y D I A L V Q E V R D S H L T A V G					
927	936	945	954	963	972
AAG CTG CTG GAC AAC CTC AAT CAG GAC GCA CCA GAC ACC TAT CAC TAC GTG GTC					
---	---	---	---	---	---
K L L D N L N Q D A P D T Y H Y V V					
981	990	999	1008	1017	1026
AGT GAG CCA CTG GGA CGG AAC AGC TAT AAG GAG CGC TAC CTG TTC GTG TAC AGG					
---	---	---	---	---	---
S E P L G R N S Y K E R Y L F V Y R					
1035	1044	1053	1062	1071	1080
CCT GAC CAG GTG TCT GCG GTG GAC AGC TAC TAC TAC GAT GAT GGC TGC GAG CCC					
---	---	---	---	---	---
P D Q V S A V D S Y Y Y D D G C E P					
1089	1098	1107	1116	1125	1134
TGC GGG AAC GAC ACC TTC AAC CGA GAG CCA GCC ATT GTC AGG TTC TTC TCC CGG					
---	---	---	---	---	---
C G N D T F N R E P A I V R F F S R					
1143	1152	1161	1170	1179	1188
TTC ACA GAG GTC AGG GAG TTT GCC ATT GTT CCC CTG CAT GCG GCC CCG GGG GAC					
---	---	---	---	---	---
F T E V R E F A I V P L H A A P G D					
1197	1206	1215	1224	1233	1242

**Fig. 6(D)**  
(Sheet 2 of 3)

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```

GCA GTA GCC GAG ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT GTC CAA GAG AAA
---
A   V   A   E   I   D   A   L   Y   D   V   Y   L   D   V   Q   E   K

      1251      1260      1269      1278      1287      1296
TGG GGC TTG GAG GAC GTC ATG TTG ATG GGC GAC TTC AAT GCG GGC TGC AGC TAT
---
W   G   L   E   D   V   M   L   M   G   D   F   N   A   G   C   S   Y

      1305      1314      1323      1332      1341      1350
GTG AGA CCC TCC CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC CCC ACC TTC CAG
---
V   R   P   S   Q   W   S   S   I   R   L   W   T   S   P   T   F   Q

      1359      1368      1377      1386      1395      1404
TGG CTG ATC CCC GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG CAC TGT GCC TAT
---
W   L   I   P   D   S   A   D   T   T   A   T   P   T   H   C   A   Y

      1413      1422      1431      1440      1449      1458
GAC AGG ATC GTG GTT GCA GGG ATG CTG CTC CGA GGG GCC GTT GTT CCC GAC TCG
---
D   R   I   V   V   A   G   M   L   L   R   G   A   V   V   P   D   S

      1467      1476      1485      1494      1503      1512
GCT CTT CCC TTT AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC CAA CTG GCC CAA
---
A   L   P   F   N   F   Q   A   A   Y   G   L   S   D   Q   L   A   Q

      1521      1530      1539      1548      1557      1566
GCC ATC AGT GAC CAC TAT CCA GTG GAG GTG ATG CTG AAG GGG GGC GGA CCC AAA
---
A   I   S   D   H   Y   P   V   E   V   M   L   K   G   G   G   P   K

      1575      1584
AAG AAG CGC AAG GTT TGA 3'
---
K   K   R   K   V   *

```

***Fig. 6D***  
***(Sheet 3 of 3)***

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## pAS34

LOCUS PAS34.DNA 2196 bp 2196 bp 2196 bp DNA 14-AUG-1998  
 DEFINITION HUMANISED HMFG1 heavy chain fused to human DNase construct 34  
 DEFINITION Clone 16.4.2 (same as hcdnasel.dna template file)  
 REFERENCE  
 AUTHORS VERHOEYEN ET AL  
 TITLE CONSTRUCTION OF RESHAPED HMFG1 etc  
 JOURNAL IMMUNOL. (1993):78, 364-370  
 COMMENT Human DNase sequence is modified as a result of oligo assembly (mhdnase.dna)  
 COMMENT The fusion was made using overlapping oligos AS79 and AS80  
 FEATURES AA RESIDUE 235 HAS NOT BEEN CHANGED TO KABAT (I.E. V TO A)  
 FEATURES Residue 963 is G > T leading to silent mutation in all clones  
 SITES Note  
 BASE COUNT 501 a 677 c 607 g 411 t  
 ORIGIN ?

```

1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
121 TGCAAGGCTT CTGGCTACAC CTTCAAGTGC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATAACAAT
241 GAGAAAGTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
361 TTTGCCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
481 GCCCTGGGCT GCCTGGTCAA GGAATACTTC CCCGAACCGG TGACGGTGTC GTGGAAGTCA
541 GCGGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCTCTC AGGACTCTAC
601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
721 GACAAAATC ACACATGCCC ACCGTGCCCC GCACCTGAAC TCCTGGGGGG ACCGTCTAGT
781 TTCCTCTTCC CCCCAAAACC CAAGGACACC CTCATGATCT CCCGGACCCG TGAGGTACAC
841 TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC
901 GGCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC
961 CGTGTGGTCA GCGTCCTCAC CGTCCTGCAC CAGGACTGGC TGAATGGCAA GGAGTACAAG
1021 TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA
1081 GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCCGGGATGA GCTGACCAAG
1141 AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG
1201 TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT GCTGGACTCC
1261 GACGGCTCCT TCTTCTCTA CAGCAAGCTC ACCGTGGACA AGAGCAGGTG GCAGCAGGGG
1321 AACGTCTTCT CATGCTCCGT CATGATGATG GCTCTGCACA ACCACTACAC GCAGAAGAGC
1381 CTCTCCCTGT CTCCGGGTAA AGGGAGCGGC GGGCTGAAGA TCGCAGCCTT CAACATCCAG
1441 ACATTTGGGG AGACCAAGAT GTCCAATGCC ACCCTCGTCA GCTACATTGT GCAGATCCTG
1501 AGCCGCTACG ACATCGCCCT GGTCCAGGAG GTCAGAGACA GCCACCTGAC TGCCGTGGGG
1561 AAGCTGCTGG ACAACCTCAA TCAGGACGCA CCAGACACCT ATCACTACGT GGTCAGTGAG
1621 CCACTGGGAC GGAACAGCTA TAAGGAGCGC TACCTGTTCTG TGTACAGGCC TGACCAGGTG
1681 TCTGCGGTGG ACAGCTACTA CTACGATGAT GGCTGCGAGC CCTGCGGGAA CGACACCTTC
1741 AACCAGAGAG CAGCCATTGT CAGGTTCTTC TCCCGGTTCA CAGAGGTCAG GGAGTTTGCC
1801 ATTGTTCCCC TGCATGCGGC CCCGGGGGAC GCAGTAGCCG AGATCGACGC TCTCTATGAC
1861 GTCTACCTGG ATGTCCAAGA GAAATGGGGC TTGGAGGACG TCATGTTGAT GGGCGACTTC
1921 AATGCGGGCT GCAGCTATGT GAGACCCCTC CAGTGGTCAT CCATCCGCCT GTGGACAAGC
1981 CCCACCTTCC AGTGGCTGAT CCCCAGACAGC GCTGACACCA CAGCTACACC CACGCACTGT
2041 GCCTATGACA GGATCGTGGT TGCAGGGATG CTGCTCCGAG GGGCCGTTGT TCCCGACTCG
2101 GCTCTTCCCT TTAACCTCCA GGCTGCCTAT GGCCTGAGTG ACCAACTGGC CCAAGCCATC
2161 AGTGACCACT ATCCAGTGGA GGTGATGCTG AAGTGA

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**Fig. 7(A)**

	9				18				27				36				45				54			
5'	ATG	GGA	TGG	AGC	TGT	ATC	ATC	CTC	TTC	TTG	GTA	GCA	ACA	GCT	ACA	GGT	GTC	CAC						
	M	G	W	S	C	I	I	L	F	L	V	A	T	A	T	G	V	H						
	63				72				81				90				99				108			
	TCC	CAG	GTG	CAG	CTG	GTG	CAG	TCT	GGG	GCA	GAG	GTG	AAA	AAG	CCT	GGG	GCC	TCA						
	<u>S</u>	Q	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S						
	117				126				135				144				153				162			
	GTG	AAG	GTG	TCC	TGC	AAG	GCT	TCT	GGC	TAC	ACC	TTC	AGT	GCC	TAC	TGG	ATA	GAG						
	V	K	V	S	C	K	A	S	G	Y	T	F	S	A	Y	W	I	E						
	171				180				189				198				207				216			
	TGG	GTG	CGC	CAG	GCT	CCA	GGA	AAG	GGC	CTC	GAG	TGG	GTC	GGA	GAG	ATT	TTA	CCT						
	W	V	R	Q	A	P	G	K	G	L	E	W	V	G	E	I	L	P						
	225				234				243				252				261				270			
	GGA	AGT	AAT	AAT	TCT	AGA	TAC	AAT	GAG	AAG	TTC	AAG	GGC	CGA	GTG	ACA	GTC	ACT						
	G	S	N	N	S	R	Y	N	E	K	F	K	G	R	V	T	V	T						
	279				288				297				306				315				324			
	AGA	GAC	ACA	TCC	ACA	AAC	ACA	GCC	TAC	ATG	GAG	CTC	AGC	AGC	CTG	AGG	TCT	GAG						
	R	D	T	S	T	N	T	A	Y	M	E	L	S	S	L	R	S	E						
	333				342				351				360				369				378			
	GAC	ACA	GCC	GTC	TAT	TAC	TGT	GCA	AGA	TCC	TAC	GAC	TTT	GCC	TGG	TTT	GCT	TAC						
	D	T	A	V	Y	Y	C	A	R	S	Y	D	F	A	W	F	A	Y						
	387				396				405				414				423				432			
	TGG	GGC	CAA	GGG	ACT	CTG	GTC	ACA	GTC	TCC	TCA	GCC	TCC	ACC	AAG	GGC	CCA	TCG						
	W	G	Q	G	T	L	V	T	V	S	S	A	S	T	K	G	P	S						
	441				450				459				468				477				486			
	GTC	TTC	CCC	CTG	GCA	CCC	TCC	TCC	AAG	AGC	ACC	TCT	GGG	GGC	ACA	GCG	GCC	CTG						
	V	F	P	L	A	P	S	S	K	S	T	S	G	G	T	A	A	L						
	495				504				513				522				531				540			
	GGC	TGC	CTG	GTC	AAG	GAC	TAC	TTC	CCC	GAA	CCG	GTG	ACG	GTG	TCG	TGG	AAC	TCA						
	G	C	L	V	K	D	Y	F	P	E	P	V	T	V	S	W	N	S						
	549				558				567				576				585				594			
	GGC	GCC	CTG	ACC	AGC	GGC	GTG	CAC	ACC	TTC	CCG	GCT	GTC	CTA	CAG	TCC	TCA	GGA						
	G	A	L	T	S	G	V	H	T	F	P	A	V	L	Q	S	S	G						
	603				612				621				630				639				648			
	CTC	TAC	TCC	CTC	AGC	AGC	GTG	GTG	ACC	GTG	CCC	TCC	AGC	AGC	TTG	GGC	ACC	CAG						
	L	Y	S	L	S	S	V	V	T	V	P	S	S	S	L	G	T	Q						
	657				666				675				684				693				702			

**Fig. 7(B)**  
(Sheet 1 of 4)

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ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA
-----
T   Y   I   C   N   V   N   H   K   P   S   N   T   K   V   D   K   K

      711      720      729      738      747      756
GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT
-----
V   E   P   K   S   C   D   K   T   H   T   C   P   P   C   P   A   P

      765      774      783      792      801      810
GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC
-----
E   L   L   G   G   P   S   V   F   L   F   P   P   K   P   K   D   T

      819      828      837      846      855      864
CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC
-----
L   M   I   S   R   T   P   E   V   T   C   V   V   V   D   V   S   H

      873      882      891      900      909      918
GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT
-----
E   D   P   E   V   K   F   N   W   Y   V   D   G   V   E   V   H   N

      927      936      945      954      963      972
GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC
-----
A   K   T   K   P   R   E   E   Q   Y   N   S   T   Y   R   V   V   S

      981      990      999      1008      1017      1026
GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG
-----
V   L   T   V   L   H   Q   D   W   L   N   G   K   E   Y   K   C   K

      1035      1044      1053      1062      1071      1080
GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA
-----
V   S   N   K   A   L   P   A   P   I   E   K   T   I   S   K   A   K

      1089      1098      1107      1116      1125      1134
GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG
-----
G   Q   P   R   E   P   Q   V   Y   T   L   P   P   S   R   D   E   L

      1143      1152      1161      1170      1179      1188
ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC
-----
T   K   N   Q   V   S   L   T   C   L   V   K   G   F   Y   P   S   D

      1197      1206      1215      1224      1233      1242
ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG
-----
I   A   V   E   W   E   S   N   G   Q   P   E   N   N   Y   K   T   T

      1251      1260      1269      1278      1287      1296
CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG
-----
P   P   V   L   D   S   D   G   S   F   F   L   Y   S   K   L   T   V

      1305      1314      1323      1332      1341      1350
GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG
-----
D   K   S   R   W   Q   Q   G   N   V   F   S   C   S   V   M   H   E

```

**Fig. 7(B)**  
(Sheet 2 of 4)

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1359	1368	1377	1386	1395	1404
GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG GGT AAA GGG					
---	---	---	---	---	---
A L H N H Y T Q K S L S L S P G K <u>G</u>					
1413	1422	1431	1440	1449	1458
AGC GGC GGG CTG AAG ATC GCA GCC TTC AAC ATC CAG ACA TTT GGG GAG ACC AAG					
---	---	---	---	---	---
<u>S G G</u> L K I A A F N I Q T F G E T K					
1467	1476	1485	1494	1503	1512
ATG TCC AAT GCC ACC CTC GTC AGC TAC ATT GTG CAG ATC CTG AGC CGC TAC GAC					
---	---	---	---	---	---
M S N A T L V S Y I V Q I L S R Y D					
1521	1530	1539	1548	1557	1566
ATC GCC CTG GTC CAG GAG GTC AGA GAC AGC CAC CTG ACT GCC GTG GGG AAG CTG					
---	---	---	---	---	---
I A L V Q E V R D S H L T A V G K L					
1575	1584	1593	1602	1611	1620
CTG GAC AAC CTC AAT CAG GAC GCA CCA GAC ACC TAT CAC TAC GTG GTC AGT GAG					
---	---	---	---	---	---
L D N L N Q D A P D T Y H Y V V S E					
1629	1638	1647	1656	1665	1674
CCA CTG GGA CGG AAC AGC TAT AAG GAG CGC TAC CTG TTC GTG TAC AGG CCT GAC					
---	---	---	---	---	---
P L G R N S Y K E R Y L F V Y R P D					
1683	1692	1701	1710	1719	1728
CAG GTG TCT GCG GTG GAC AGC TAC TAC TAC GAT GAT GGC TGC GAG CCC TGC GGG					
---	---	---	---	---	---
Q V S A V D S Y Y Y D D G C E P C G					
1737	1746	1755	1764	1773	1782
AAC GAC ACC TTC AAC CGA GAG CCA GCC ATT GTC AGG TTC TTC TCC CGG TTC ACA					
---	---	---	---	---	---
N D T F N R E P A I V R F F S R F T					
1791	1800	1809	1818	1827	1836
GAG GTC AGG GAG TTT GCC ATT GTT CCC CTG CAT GCG GCC CCG GGG GAC GCA GTA					
---	---	---	---	---	---
E V R E F A I V P L H A A P G D A V					
1845	1854	1863	1872	1881	1890
GCC GAG ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT GTC CAA GAG AAA TGG GGC					
---	---	---	---	---	---
A E I D A L Y D V Y L D V Q E K W G					
1899	1908	1917	1926	1935	1944
TTG GAG GAC GTC ATG TTG ATG GGC GAC TTC AAT GCG GGC TGC AGC TAT GTG AGA					
---	---	---	---	---	---
L E D V M L M G D F N A G C S Y V R					
1953	1962	1971	1980	1989	1998
CCC TCC CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC CCC ACC TTC CAG TGG CTG					
---	---	---	---	---	---
P S Q W S S I R L W T S P T F Q W L					
2007	2016	2025	2034	2043	2052
ATC CCC GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG CAC TGT GCC TAT GAC AGG					
---	---	---	---	---	---
I P D S A D T T A T P T H C A Y D R					

**Fig. 7(B)**  
(Sheet 3 of 4)

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2061	2070	2079	2088	2097	2106
ATC GTG GTT GCA GGG ATG CTG CTC CGA GGG GCC GTT GTT CCC GAC TCG GCT CTT					
---	---	---	---	---	---
I V V A G M L L R G A V V P D S A L					
2115	2124	2133	2142	2151	2160
CCC TTT AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC CAA CTG GCC CAA GCC ATC					
---	---	---	---	---	---
P F N F Q A A Y G L S D Q L A Q A I					
2169	2178	2187	2196		
AGT GAC CAC TAT CCA GTG GAG GTG ATG CTG AAG TGA 3'					
---	---	---	---		
S D H Y P V E V M L K *					

***Fig. 7(B)***  
***(Sheet 4 of 4)***

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## pAS35

LOCUS PAS35.DNA 2193 bp 2193 bp DNA 14-AUG-1998  
 DEFINITION HUMANISED HMFG1 heavy chain fused to human DNase construct 35  
 DEFINITION Clone 17.12.1 with silent K to K mutation (1398 A > G)  
 REFERENCE  
 AUTHORS VERHOEYEN ET AL  
 TITLE CONSTRUCTION OF RESHAPED HMFG1 etc  
 JOURNAL IMMUNOL. (1993):78, 364-370  
 COMMENT Human DNase sequence is modified as a result of oligo assembly (mhdnase.dna)  
 COMMENT The fusion was made using overlapping oligos AS81 and AS82  
 FEATURES AA RESIDUE 235 HAS NOT BEEN CHANGED TO KABAT (I.E. V TO A)  
 FEATURES Residue 963 is G > T leading to silent mutation in all clones  
 FEATURES In 17.12.1 residue 1398 is A > G (silent K to K mutation)  
 SITES Note  
 BASE COUNT 500 a 677 c 606 g 410 t  
 ORIGIN ?

```

1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
121 TGCAAGGCTT CTGGCTACAC CTTCAAGTGC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTG TAGATACAAT
241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
361 TTTGCCCTGG TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTG GTGGAACCTCA
541 GCGGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
721 GACAAAACCT ACACATGCCC ACCGTGCCCC GCACCTGAAC TCCTGGGGGG ACCGTACGTC
781 TTCCTCTTCC CCCCCAAACC CAAGGACACC CTCATGATCT CCCGGACCCC TGAGGTACAC
841 TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC
901 GGCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC
961 CGTGTGGTCA GCGTCTTCAC CGTCTGTCAC CAGGACTGGC TGAATGGCAA GGAGTACAAG
1021 TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA
1081 GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCCGGGATGA GCTGACCAAG
1141 AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG
1201 TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT GCTGGACTCC
1261 GACGGCTCCT TCTTCTCTA CAGCAAGCTC ACCGTGGACA AGAGCAGGTG GCAGCAGGGG
1321 AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC
1381 CTCTCCCTGT CTCCGAAGGG GAGCGGCGGG CTGAAGATCG CAGCCTTCAA CATCCAGACA
1441 TTTGGGGAGA CCAAGATGTC CAATGCCACC CTCGTCAGCT ACATTGTGCA GATCCTGAGC
1501 CGCTACGACA TCGCCCTGGT CCAGGAGGTC AGAGACAGCC ACCTGACTGC CGTGGGGAAG
1561 CTGCTGGACA ACCTCAATCA GGACGCACCA GACACCTATC ACTACGTGGT CAGTGAGCCA
1621 CTGGGACGGA ACAGCTATAA GGAGCGCTAC CTGTTTCGTG ACAGGCCTGA CCAGGTGTCT
1681 GCGGTGGACA GCTACTACTA CGATGATGGC TGCGAGCCCT GCGGGAACGA CACCTTCAAC
1741 CGAGAGCCAG CCATTGTCAG GTTCTTCTCC CGGTTACAG AGGTTCAGGA GTTTGCCATT
1801 GTTCCCCTGC ATGCGGCCCC GGGGACGCA GATGCCGAGA CTAGCCGTCT CTATGAGCTC
1861 TACCTGGATG TCCAAGAGAA ATGGGGCTTG GAGGACGTCA TGTTGATGGG CGACTTCAAT
1921 GCGGGCTGCA GCTATGTGAG ACCCTCCCAG TGGTCATCCA TCCGCTGTG GACAAGCCCC
1981 ACCTTCCAGT GGCTGATCCC CGACAGCGCT GACACCACAG CTACACCCAC GCACTGTGCC
2041 TATGACAGGA TCGTGGTTGC AGGGATGCTG CTCCGAGGGG CCGTTGTTCC CGACTCGGCT
2101 CTTCCCTTTA ACTTCCAGGC TGCCTATGGC CTGAGTGACC AACTGGCCCA AGCCATCAGT
2161 GACCACTATC CAGTGGAGGT GATGCTGAAG TGA

```

//

**Fig. 8(A)**

	9			18			27			36			45			54		
5'	ATG	GGA	TGG	AGC	TGT	ATC	ATC	CTC	TTC	TTG	GTA	GCA	ACA	GCT	ACA	GGT	GTC	CAC
	M	G	W	S	C	I	I	L	F	L	V	A	T	A	T	G	V	H
	63			72			81			90			99			108		
	TCC	CAG	GTG	CAG	CTG	GTG	CAG	TCT	GGG	GCA	GAG	GTG	AAA	AAG	CCT	GGG	GCC	TCA
	S	Q	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S
	117			126			135			144			153			162		
	GTG	AAG	GTG	TCC	TGC	AAG	GCT	TCT	GGC	TAC	ACC	TTC	AGT	GCC	TAC	TGG	ATA	GAG
	V	K	V	S	C	K	A	S	G	Y	T	F	S	A	Y	W	I	E
	171			180			189			198			207			216		
	TGG	GTG	CGC	CAG	GCT	CCA	GGA	AAG	GGC	CTC	GAG	TGG	GTC	GGA	GAG	ATT	TTA	CCT
	W	V	R	Q	A	P	G	K	G	L	E	W	V	G	E	I	L	P
	225			234			243			252			261			270		
	GGA	AGT	AAT	AAT	TCT	AGA	TAC	AAT	GAG	AAG	TTC	AAG	GGC	CGA	GTG	ACA	GTC	ACT
	G	S	N	N	S	R	Y	N	E	K	F	K	G	R	V	T	V	T
	279			288			297			306			315			324		
	AGA	GAC	ACA	TCC	ACA	AAC	ACA	GCC	TAC	ATG	GAG	CTC	AGC	AGC	CTG	AGG	TCT	GAG
	R	D	T	S	T	N	T	A	Y	M	E	L	S	S	L	R	S	E
	333			342			351			360			369			378		
	GAC	ACA	GCC	GTC	TAT	TAC	TGT	GCA	AGA	TCC	TAC	GAC	TTT	GCC	TGG	TTT	GCT	TAC
	D	T	A	V	Y	Y	C	A	R	S	Y	D	F	A	W	F	A	Y
	387			396			405			414			423			432		
	TGG	GGC	CAA	GGG	ACT	CTG	GTC	ACA	GTC	TCC	TCA	GCC	TCC	ACC	AAG	GGC	CCA	TCG
	W	G	Q	G	T	L	V	T	V	S	S	A	S	T	K	G	P	S
	441			450			459			468			477			486		
	GTC	TTC	CCC	CTG	GCA	CCC	TCC	TCC	AAG	AGC	ACC	TCT	GGG	GGC	ACA	GCG	GCC	CTG
	V	F	P	L	A	P	S	S	K	S	T	S	G	G	T	A	A	L
	495			504			513			522			531			540		
	GGC	TGC	CTG	GTC	AAG	GAC	TAC	TTC	CCC	GAA	CCG	GTG	ACG	GTG	TCG	TGG	AAC	TCA
	G	C	L	V	K	D	Y	F	P	E	P	V	T	V	S	W	N	S
	549			558			567			576			585			594		
	GGC	GCC	CTG	ACC	AGC	GGC	GTG	CAC	ACC	TTC	CCG	GCT	GTC	CTA	CAG	TCC	TCA	GGA
	G	A	L	T	S	G	V	H	T	F	P	A	V	L	Q	S	S	G
	603			612			621			630			639			648		
	CTC	TAC	TCC	CTC	AGC	AGC	GTG	GTG	ACC	GTG	CCC	TCC	AGC	AGC	TTG	GGC	ACC	CAG
	L	Y	S	L	S	S	V	V	T	V	P	S	S	S	L	G	T	Q
	657			666			675			684			693			702		

**Fig. 8(B)**  
(Sheet 1 of 4)

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ACC	TAC	ATC	TGC	AAC	GTG	AAT	CAC	AAG	CCC	AGC	AAC	ACC	AAG	GTG	GAC	AAG	AAA				
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---				
T	Y	I	C	N	V	N	H	K	P	S	N	T	K	V	D	K	K				
711				720				729				738				747				756	
GTT	GAG	CCC	AAA	TCT	TGT	GAC	AAA	ACT	CAC	ACA	TGC	CCA	CCG	TGC	CCA	GCA	CCT				
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---				
V	E	P	K	S	C	D	K	T	H	T	C	P	P	C	P	A	P				
765				774				783				792				801				810	
GAA	CTC	CTG	GGG	GGA	CCG	TCA	GTC	TTC	CTC	TTC	CCC	CCA	AAA	CCC	AAG	GAC	ACC				
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---				
E	L	L	G	G	P	S	V	F	L	F	P	P	K	P	K	D	T				
819				828				837				846				855				864	
CTC	ATG	ATC	TCC	CGG	ACC	CCT	GAG	GTC	ACA	TGC	GTG	GTG	GTG	GAC	GTG	AGC	CAC				
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---				
L	M	I	S	R	T	P	E	V	T	C	V	V	V	D	V	S	H				
873				882				891				900				909				918	
GAA	GAC	CCT	GAG	GTC	AAG	TTC	AAC	TGG	TAC	GTG	GAC	GGC	GTG	GAG	GTG	CAT	AAT				
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---				
E	D	P	E	V	K	F	N	W	Y	V	D	G	V	E	V	H	N				
927				936				945				954				963				972	
GCC	AAG	ACA	AAG	CCG	CGG	GAG	GAG	CAG	TAC	AAC	AGC	ACG	TAC	CGT	GTG	GTC	AGC				
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---				
A	K	T	K	P	R	E	E	Q	Y	N	S	T	Y	R	V	V	S				
981				990				999				1008				1017				1026	
GTC	CTC	ACC	GTC	CTG	CAC	CAG	GAC	TGG	CTG	AAT	GGC	AAG	GAG	TAC	AAG	TGC	AAG				
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---				
V	L	T	V	L	H	Q	D	W	L	N	G	K	E	Y	K	C	K				
1035				1044				1053				1062				1071				1080	
GTC	TCC	AAC	AAA	GCC	CTC	CCA	GCC	CCC	ATC	GAG	AAA	ACC	ATC	TCC	AAA	GCC	AAA				
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---				
V	S	N	K	A	L	P	A	P	I	E	K	T	I	S	K	A	K				
1089				1098				1107				1116				1125				1134	
GGG	CAG	CCC	CGA	GAA	CCA	CAG	GTG	TAC	ACC	CTG	CCC	CCA	TCC	CGG	GAT	GAG	CTG				
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---				
G	Q	P	R	E	P	Q	V	Y	T	L	P	P	S	R	D	E	L				
1143				1152				1161				1170				1179				1188	
ACC	AAG	AAC	CAG	GTC	AGC	CTG	ACC	TGC	CTG	GTC	AAA	GGC	TTC	TAT	CCC	AGC	GAC				
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---				
T	K	N	Q	V	S	L	T	C	L	V	K	G	F	Y	P	S	D				
1197				1206				1215				1224				1233				1242	
ATC	GCC	GTG	GAG	TGG	GAG	AGC	AAT	GGG	CAG	CCG	GAG	AAC	AAC	TAC	AAG	ACC	ACG				
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---				
I	A	V	E	W	E	S	N	G	Q	P	E	N	N	Y	K	T	T				
1251				1260				1269				1278				1287				1296	
CCT	CCC	GTG	CTG	GAC	TCC	GAC	GGC	TCC	TTC	TTC	CTC	TAC	AGC	AAG	CTC	ACC	GTG				
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---				
P	P	V	L	D	S	D	G	S	F	F	L	Y	S	K	L	T	V				
1305				1314				1323				1332				1341				1350	
GAC	AAG	AGC	AGG	TGG	CAG	CAG	GGG	AAC	GTC	TTC	TCA	TGC	TCC	GTG	ATG	CAT	GAG				
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---				
D	K	S	R	W	Q	Q	G	N	V	F	S	C	S	V	M	H	E				

**Fig. 8(B)**  
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1359	1368	1377	1386	1395	1404
GCT CTG CAC AAC CAC TAC	ACG CAG AAG AGC	CTC TCC CTG TCT CCG AAG	GGG AGC		
A L H N H Y T Q K S L S L S P K	<u>G</u> <u>S</u>				
1413	1422	1431	1440	1449	1458
GGC GGG CTG AAG ATC GCA GCC TTC AAC ATC	CAG ACA TTT GGG GAG ACC AAG ATG				
<u>G</u> <u>G</u> L K I A A F N I Q T F G E T K M					
1467	1476	1485	1494	1503	1512
TCC AAT GCC ACC CTC GTC AGC TAC ATT GTG	CAG ATC CTG AGC CGC TAC GAC ATC				
S N A T L V S Y I V Q I L S R Y D I					
1521	1530	1539	1548	1557	1566
GCC CTG GTC CAG GAG GTC AGA GAC AGC CAC	CTG ACT GCC GTG GGG AAG CTG CTG				
A L V Q E V R D S H L T A V G K L L					
1575	1584	1593	1602	1611	1620
GAC AAC CTC AAT CAG GAC GCA CCA GAC ACC	TAT CAC TAC GTG GTC AGT GAG CCA				
D N L N Q D A P D T Y H Y V V S E P					
1629	1638	1647	1656	1665	1674
CTG GGA CGG AAC AGC TAT AAG GAG CGC TAC	CTG TTC GTG TAC AGG CCT GAC CAG				
L G R N S Y K E R Y L F V Y R P D Q					
1683	1692	1701	1710	1719	1728
GTG TCT GCG GTG GAC AGC TAC TAC TAC GAT	GAT GGC TGC GAG CCC TGC GGG AAC				
V S A V D S Y Y Y D D G C E P C G N					
1737	1746	1755	1764	1773	1782
GAC ACC TTC AAC CGA GAG CCA GCC ATT GTC	AGG TTC TTC TCC CGG TTC ACA GAG				
D T F N R E P A I V R F F S R F T E					
1791	1800	1809	1818	1827	1836
GTC AGG GAG TTT GCC ATT GTT CCC CTG CAT	GCG GCC CCG GGG GAC GCA GTA GCC				
V R E F A I V P L H A A P G D A V A					
1845	1854	1863	1872	1881	1890
GAG ATC GAC GCT CTC TAT GAC GTC TAC CTG	GAT GTC CAA GAG AAA TGG GGC TTG				
E I D A L Y D V Y L D V Q E K W G L					
1899	1908	1917	1926	1935	1944
GAG GAC GTC ATG TTG ATG GGC GAC TTC AAT	GCG GGC TGC AGC TAT GTG AGA CCC				
E D V M L M G D F N A G C S Y V R P					
1953	1962	1971	1980	1989	1998
TCC CAG TGG TCA TCC ATC CGC CTG TGG ACA	AGC CCC ACC TTC CAG TGG CTG ATC				
S Q W S S I R L W T S P T F Q W L I					
2007	2016	2025	2034	2043	2052
CCC GAC AGC GCT GAC ACC ACA GCT ACA CCC	ACG CAC TGT GCC TAT GAC AGG ATC				
P D S A D T T A T P T H C A Y D R I					

**Fig. 8(B)**  
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2061	2070	2079	2088	2097	2106
GTG GTT GCA GGG ATG CTG CTC CGA GGG GCC GTT GTT CCC GAC TCG GCT CTT CCC					
-----	-----	-----	-----	-----	-----
V V A G M L L R G A V V P D S A L P					
2115	2124	2133	2142	2151	2160
TTT AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC CAA CTG GCC CAA GCC ATC AGT					
-----	-----	-----	-----	-----	-----
F N F Q A A Y G L S D Q L A Q A I S					
2169	2178	2187			
GAC CAC TAT CCA GTG GAG GTG ATG CTG AAG TGA 3'					
-----	-----	-----			
D H Y P V E V M L K *					

***Fig. 8(B)***  
***(Sheet 4 of 4)***

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## pAS36

LOCUS PAS36.DNA 2190 bp 2190 bp DNA 14-AUG-1998

DEFINITION HUMANISED HMFG1 heavy chain fused to human DNase - construct 36

DEFINITION Clone 18.24.1 with residue 1392 T > C

REFERENCE

AUTHORS VERHOEYEN ET AL

TITLE CONSTRUCTION OF RESHAPED HMFG1 etc

JOURNAL IMMUNOL. (1993):78, 364-370

COMMENT Human DNase sequence is modified as a result of oligo assembly (mhdnase.dna)

COMMENT The fusion was made using overlapping oligos AS83 and AS84

FEATURES AA RESIDUE 235 HAS NOT BEEN CHANGED TO KABAT (I.E. V TO A)

FEATURES Residue 963 is G > T leading to silent mutation in all clones

FEATURES Residue 1392 T > C silent S to S mutation

SITES Note

BASE COUNT 498 a 678 c 605 g 409 t

ORIGIN ?

```

1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
121 TGCAAGGCTT CTGGCTACAC CTTCAAGTGC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACCTCA
541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
721 GACAAAACTC ACACATGCCC ACCGTGCCCA GCACCTGAAC TCCTGGGGGG ACCGTCAGTC
781 TTCCTCTTCC CCCCCAAAACC CAAGGACACC CTCATGATCT CCCGGACCCC TGAGGTCACA
841 TCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC
901 GCGTGGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC
961 CGTGTGGTCA GCGTCCTCAC CGTCCTGCAC CAGGACTGGC TGAATGGCAA GGAGTACAAG
1021 TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA
1081 GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCCGGGATGA GCTGACCAAG
1141 AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG
1201 TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT GCTGGACTCC
1261 GACGGCTCCT TCTTCCTCTA CAGCAAGCTC ACCGTGGACA AGAGCAGGTG GCAGCAGGGG
1321 AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC
1381 CTCTCCCTGT CcCCGGGGAG CGGCGGGCTG AAGATCGCAG CCTTCAACAT CCAGACATTT
1441 GGGGAGACCA AGATGTCCAA TGCCACCCTC GTCAGCTACA TTGTGCAGAT CCTGAGCCGC
1501 TACGACATCG CCCTGGTCCA GGAGGTCAGA GACAGCCACC TGACTGCCGT GGGGAAGCTG
1561 CTGGACAACC TCAATCAGGA CGCACCAGAC ACCTATCACT ACGTGGTCAG TGAGCCACTG
1621 GGACGGAACA GCTATAAGGA GCGTACCTG TTCGTGTACA GGCCTGACCA GGTGTCTGCG
1681 GTGGACAGCT ACTACTACGA TGATGGCTGC GAGCCCTGCG GGAACGACAC CTTCAACCGA
1741 GAGCCAGCCA TTGTCAAGTT CTCTCTCCCG TTCACAGAGG TCAGGGAGTT TGCCATTGTT
1801 CCCCTGCATG CGGCCCCGGG GGCAGCAGTA CCGGAGATCG ACGCTCTCTA TGACGTCTAC
1861 CTGGATGTCC AAGAGAAAATG GGGCTTGGAG GACGTCATGT TGATGGGCGA CTTCAATGCG
1921 GGCTGCAGCT ATGTGAGACC CTCCCAGTGG TCATCCATCC GCCTGTGGAC AAGCCCCACC
1981 TTCCAGTGGC TGATCCCCGA CAGCGCTGAC ACCACAGCTA CACCCACGCA CTGTGCCTAT
2041 GACAGGATCG TGGTTGCAGG GATGCTGCTC CGAGGGGCCG TTGTTCCCGA CTCGGCTCTT
2101 CCCTTTAACT TCCAGGCTGC CTATGGCCTG AGTGACCAAC TGGCCCAAGC CATCAGTGAC
2161 CACTATCCAG TGGAGGTGAT GCTGAAGTGA

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**Fig. 9(A)**

5'	9				18				27				36				45				54	
	ATG	GGA	TGG	AGC	TGT	ATC	ATC	CTC	TTC	TTG	GTA	GCA	ACA	GCT	ACA	GGT	GTC	CAC				
	M	G	W	S	C	I	I	L	F	L	V	A	T	A	T	G	V	H				
	63				72				81				90				99				108	
	TCC	CAG	GTC	CAG	CTG	GTG	CAG	TCT	GGG	GCA	GAG	GTG	AAA	AAG	CCT	GGG	GCC	TCA				
	S	Q	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S				
	117				126				135				144				153				162	
	GTG	AAG	GTG	TCC	TGC	AAG	GCT	TCT	GGC	TAC	ACC	TTC	AGT	GCC	TAC	TGG	ATA	GAG				
	V	K	V	S	C	K	A	S	G	Y	T	F	S	A	Y	W	I	E				
	171				180				189				198				207				216	
	TGG	GTG	CGC	CAG	GCT	CCA	GGA	AAG	GGC	CTC	GAG	TGG	GTC	GGA	GAG	ATT	TTA	CCT				
	W	V	R	Q	A	P	G	K	G	L	E	W	V	G	E	I	L	P				
	225				234				243				252				261				270	
	GGA	AGT	AAT	AAT	TCT	AGA	TAC	AAT	GAG	AAG	TTC	AAG	GGC	CGA	GTG	ACA	GTC	ACT				
	G	S	N	N	S	R	Y	N	E	K	F	K	G	R	V	T	V	T				
	279				288				297				306				315				324	
	AGA	GAC	ACA	TCC	ACA	AAC	ACA	GCC	TAC	ATG	GAG	CTC	AGC	AGC	CTG	AGG	TCT	GAG				
	R	D	T	S	T	N	T	A	Y	M	E	L	S	S	L	R	S	E				
	333				342				351				360				369				378	
	GAC	ACA	GCC	GTC	TAT	TAC	TGT	GCA	AGA	TCC	TAC	GAC	TTT	GCC	TGG	TTT	GCT	TAC				
	D	T	A	V	Y	Y	C	A	R	S	Y	D	F	A	W	F	A	Y				
	387				396				405				414				423				432	
	TGG	GGC	CAA	GGG	ACT	CTG	GTC	ACA	GTC	TCC	TCA	GCC	TCC	ACC	AAG	GGC	CCA	TCG				
	W	G	Q	G	T	L	V	T	V	S	S	A	S	T	K	G	P	S				
	441				450				459				468				477				486	
	GTC	TTC	CCC	CTG	GCA	CCC	TCC	TCC	AAG	AGC	ACC	TCT	GGG	GGC	ACA	GCG	GCC	CTG				
	V	F	P	L	A	P	S	S	K	S	T	S	G	G	T	A	A	L				
	495				504				513				522				531				540	
	GGC	TGC	CTG	GTC	AAG	GAC	TAC	TTC	CCC	GAA	CCG	GTG	ACG	GTG	TCG	TGG	AAC	TCA				
	G	C	L	V	K	D	Y	F	P	E	P	V	T	V	S	W	N	S				
	549				558				567				576				585				594	
	GGC	GCC	CTG	ACC	AGC	GGC	GTG	CAC	ACC	TTC	CCG	GCT	GTC	CTA	CAG	TCC	TCA	GGA				
	G	A	L	T	S	G	V	H	T	F	P	A	V	L	Q	S	S	G				
	603				612				621				630				639				648	
	CTC	TAC	TCC	CTC	AGC	AGC	GTG	GTG	ACC	GTG	CCC	TCC	AGC	AGC	TTG	GGC	ACC	CAG				
	L	Y	S	L	S	S	V	V	T	V	P	S	S	S	L	G	T	Q				
	657				666</																	

***Fig. 9(B)***  
***(Sheet 1 of 4)***

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ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA
-----
T   Y   I   C   N   V   N   H   K   P   S   N   T   K   V   D   K   K

      711      720      729      738      747      756
GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT
-----
V   E   P   K   S   C   D   K   T   H   T   C   P   P   C   P   A   P

      765      774      783      792      801      810
GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC
-----
E   L   L   G   G   P   S   V   F   L   F   P   P   K   P   K   D   T

      819      828      837      846      855      864
CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC
-----
L   M   I   S   R   T   P   E   V   T   C   V   V   V   D   V   S   H

      873      882      891      900      909      918
GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT
-----
E   D   P   E   V   K   F   N   W   Y   V   D   G   V   E   V   H   N

      927      936      945      954      963      972
GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC
-----
A   K   T   K   P   R   E   E   Q   Y   N   S   T   Y   R   V   V   S

      981      990      999      1008      1017      1026
GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG
-----
V   L   T   V   L   H   Q   D   W   L   N   G   K   E   Y   K   C   K

      1035      1044      1053      1062      1071      1080
GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA
-----
V   S   N   K   A   L   P   A   P   I   E   K   T   I   S   K   A   K

      1089      1098      1107      1116      1125      1134
GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG
-----
G   Q   P   R   E   P   Q   V   Y   T   L   P   P   S   R   D   E   L

      1143      1152      1161      1170      1179      1188
ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC
-----
T   K   N   Q   V   S   L   T   C   L   V   K   G   F   Y   P   S   D

      1197      1206      1215      1224      1233      1242
ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG
-----
I   A   V   E   W   E   S   N   G   Q   P   E   N   N   Y   K   T   T

      1251      1260      1269      1278      1287      1296
CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG
-----
P   P   V   L   D   S   D   G   S   F   F   L   Y   S   K   L   T   V

      1305      1314      1323      1332      1341      1350
GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG
-----
D   K   S   R   W   Q   Q   G   N   V   F   S   C   S   V   M   H   E

```

**Fig. 9(B)**  
(Sheet 2 of 4)

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1359	1368	1377	1386	1395	1404
GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCC CCG GGG AGC GGC					
A L H N H Y T Q K S L S L S P <u>G S G</u>					
1413	1422	1431	1440	1449	1458
GGG CTG AAG ATC GCA GCC TTC AAC ATC CAG ACA TTT GGG GAG ACC AAG ATG TCC					
<u>G</u> L K I A A F N I Q T F G E T K M S					
1467	1476	1485	1494	1503	1512
AAT GCC ACC CTC GTC AGC TAC ATT GTG CAG ATC CTG AGC CGC TAC GAC ATC GCC					
N A T L V S Y I V Q I L S R Y D I A					
1521	1530	1539	1548	1557	1566
CTG GTC CAG GAG GTC AGA GAC AGC CAC CTG ACT GCC GTG GGG AAG CTG CTG GAC					
L V Q E V R D S H L T A V G K L L D					
1575	1584	1593	1602	1611	1620
AAC CTC AAT CAG GAC GCA CCA GAC ACC TAT CAC TAC GTG GTC AGT GAG CCA CTG					
N L N Q D A P D T Y H Y V V S E P L					
1629	1638	1647	1656	1665	1674
GGA CGG AAC AGC TAT AAG GAG CGC TAC CTG TTC GTG TAC AGG CCT GAC CAG GTG					
G R N S Y K E R Y L F V Y R P D Q V					
1683	1692	1701	1710	1719	1728
TCT GCG GTG GAC AGC TAC TAC TAC GAT GAT GGC TGC GAG CCC TGC GGG AAC GAC					
S A V D S Y Y Y D D G C E P C G N D					
1737	1746	1755	1764	1773	1782
ACC TTC AAC CGA GAG CCA GCC ATT GTC AGG TTC TTC TCC CGG TTC ACA GAG GTC					
T F N R E P A I V R F F S R F T E V					
1791	1800	1809	1818	1827	1836
AGG GAG TTT GCC ATT GTT CCC CTG CAT GCG GCC CCG GGG GAC GCA GTA GCC GAG					
R E F A I V P L H A A P G D A V A E					
1845	1854	1863	1872	1881	1890
ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT GTC CAA GAG AAA TGG GGC TTG GAG					
I D A L Y D V Y L D V Q E K W G L E					
1899	1908	1917	1926	1935	1944
GAC GTC ATG TTG ATG GGC GAC TTC AAT GCG GGC TGC AGC TAT GTG AGA CCC TCC					
D V M L M G D F N A G C S Y V R P S					
1953	1962	1971	1980	1989	1998
CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC CCC ACC TTC CAG TGG CTG ATC CCC					
Q W S S I R L W T S P T F Q W L I P					
2007	2016	2025	2034	2043	2052
GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG CAC TGT GCC TAT GAC AGG ATC GTG					
D S A D T T A T P T H C A Y D R I V					

**Fig. 9(B)**  
**(Sheet 3 of 4)**

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2061	2070	2079	2088	2097	2106
GTT GCA GGG ATG CTG CTC CGA GGG GCC GTT GTT CCC GAC TCG GCT CTT CCC TTT					
---	---	---	---	---	---
V A G M L L R G A V V P D S A L P F					
2115	2124	2133	2142	2151	2160
AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC CAA CTG GCC CAA GCC ATC AGT GAC					
---	---	---	---	---	---
N F Q A A Y G L S D Q L A Q A I S D					
2169	2178	2187			
CAC TAT CCA GTG GAG GTG ATG CTG AAG TGA 3'					
---	---	---			
H Y P V E V M L K *					

***Fig. 9(B)***  
***(Sheet 4 of 4)***

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## pAS37

LOCUS PAS37.DNA 2226 bp 2196 bp 2196 bp DNA 14-AUG-1998

DEFINITION HUMANISED HMFG1 heavy chain fused to human DNase construct 37

DEFINITION Clone 16.4.2 (same as hcdnase1.dna template file) plus NLS

REFERENCE

AUTHORS VERHOEYEN ET AL

TITLE CONSTRUCTION OF RESHAPED HMFG1 etc

JOURNAL IMMUNOL. (1993):78, 364-370

COMMENT Human DNase sequence is modified as a result of oligo assembly (mhdnase.dna)

COMMENT The fusion was made using overlapping oligos AS79 and AS80

FEATURES AA RESIDUE 235 HAS NOT BEEN CHANGED TO KABAT (I.E. V TO A)

FEATURES Residue 963 is G > T leading to silent mutation in all clones

SITES Note

BASE COUNT 511 a 683 c 619 g 413 t


ORIGIN ?

```

1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
121 TGCAAGGCTT CTGGCTACAC CTTCACTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAAC TCA
541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCTCTC AGGACTCTAC
601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAG CTACATCTGC
661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
721 GACAAAAC TCACATGCCC ACCGTGCCCA GCACCTGAAC TCCTGGGGGG ACCGTAGTC
781 TTCCTCTTCC CCCCAAAACC CAAGGACACC CTCATGATCT CCCGGACCCC TGAGGTCACA
841 TCGCTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC
901 GCGCTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC
961 CGTGTGGTCA GCGTCCTCAC CGTCCTGCAC CAGGACTGGC TGAATGGCAA GGAGTACAAG
1021 TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA
1081 GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCCGGGATGA GCTGACCAAG
1141 AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG
1201 TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT GCTGGACTCC
1261 GACGGCTCCT TCTTCTCTTA CAGCAAGCTC ACCGTGGACA AGACGAGGTG GCAGCAGGGG
1321 AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC
1381 CTCTCCCTGT CTCCGGGTAA AGGGAGCGGC GGGCTGAAGA TCGCAGCCTT CAACATCCAG
1441 ACATTTGGGG AGACCAAGAT GTCCAATGCC ACCCTCGTCA GCTACATTGT GCAGATCCTG
1501 AGCCGCTACG ACATCGCCCT GGTCCAGGAG GTCAGAGACA GCCACCTGAC TGCCGTGGGG
1561 AAGCTGCTGG ACAACCTCAA TCAGGACGCA CCAGACACCT ATCACTACGT GGTCACTGAG
1621 CCACTGGGAC GGAACAGCTA TAAGGAGCGC TACCTGTTTC TGTACAGGCC TGACCAGGTG
1681 TCTGCGGTGG ACAGCTACTA CTACGATGAT GGCTGCGAGC CCTGCGGGAA CGACACCTTC
1741 AACCAGAGAG CAGCCATTGT CAGGTTCTTC TCCCGTTTCA CAGAGGTCAG GGAGTTTGCC
1801 ATTGTTCCCC TGCATGCGGC CCCGGGGGAC CGAGTAGCCG AGATGACGAC TCTCTATGAC
1861 GTCTACCTGG ATGTCCAAGA GAAATGGGGC TTGGAGGACG TCATGTTGAT GGGCGACTTC
1921 AATGCGGGCT GCAGCTATGT GAGACCCTCC CAGTGGTCAT CCATCCGCCT GTGGACAAGC
1981 CCCACCTTCC AGTGGCTGAT CCCCAGACAG GCTGACACCA CAGCTACACC CACGCACTGT
2041 GCCTATGACA GGATCGTGGT TGCAGGGATG CTGCTCCGAG GGGCCGTTGT TCCCGACTCG
2101 GCTCTTCCCT TTAACCTCCA GGCTGCCTAT GGCCTGAGTG ACCAACTGGC CCAAGCCATC
2161 AGTGACCACT ATCCAGTGGA GGTGATGCTG AAGGGGGGCG GACCCAAAAA GAAGCGCAAG
2221 GTTTGA

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//



**Fig. 10(A)**

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	9				18				27				36				45				54			
5'	ATG	GGA	TGG	AGC	TGT	ATC	ATC	CTC	TTC	TTG	GTA	GCA	ACA	GCT	ACA	GGT	GTC	CAC						
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---						
	M	G	W	S	C	I	I	L	F	L	V	A	T	A	T	G	V	H						
			63			72			81			90			99			108						
	TCC	CAG	GTG	CAG	CTG	GTG	CAG	TCT	GGG	GCA	GAG	GTG	AAA	AAG	CCT	GGG	GCC	TCA						
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---						
	<u>S</u>	Q	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S						
			117			126			135			144			153			162						
	GTG	AAG	GTG	TCC	TGC	AAG	GCT	TCT	GGC	TAC	ACC	TTC	AGT	GCC	TAC	TGG	ATA	GAG						
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---						
	V	K	V	S	C	K	A	S	G	Y	T	F	S	A	Y	W	I	E						
			171			180			189			198			207			216						
	TGG	GTG	CGC	CAG	GCT	CCA	GGA	AAG	GGC	CTC	GAG	TGG	GTC	GGA	GAG	ATT	TTA	CCT						
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---						
	W	V	R	Q	A	P	G	K	G	L	E	W	V	G	E	I	L	P						
			225			234			243			252			261			270						
	GGA	AGT	AAT	AAT	TCT	AGA	TAC	AAT	GAG	AAG	TTC	AAG	GGC	CGA	GTG	ACA	GTC	ACT						
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---						
	G	S	N	N	S	R	Y	N	E	K	F	K	G	R	V	T	V	T						
			279			288			297			306			315			324						
	AGA	GAC	ACA	TCC	ACA	AAC	ACA	GCC	TAC	ATG	GAG	CTC	AGC	AGC	CTG	AGG	TCT	GAG						
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---						
	R	D	T	S	T	N	T	A	Y	M	E	L	S	S	L	R	S	E						
			333			342			351			360			369			378						
	GAC	ACA	GCC	GTC	TAT	TAC	TGT	GCA	AGA	TCC	TAC	GAC	TTT	GCC	TGG	TTT	GCT	TAC						
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---						
	D	T	A	V	Y	Y	C	A	R	S	Y	D	F	A	W	F	A	Y						
			387			396			405			414			423			432						
	TGG	GGC	CAA	GGG	ACT	CTG	GTC	ACA	GTC	TCC	TCA	GCC	TCC	ACC	AAG	GGC	CCA	TCG						
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---						
	W	G	Q	G	T	L	V	T	V	S	S	A	S	T	K	G	P	S						
			441			450			459			468			477			486						
	GTC	TTC	CCC	CTG	GCA	CCC	TCC	TCC	AAG	AGC	ACC	TCT	GGG	GGC	ACA	GCG	GCC	CTG						
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---						
	V	F	P	L	A	P	S	S	K	S	T	S	G	G	T	A	A	L						
			495			504			513			522			531			540						
	GGC	TGC	CTG	GTC	AAG	GAC	TAC	TTC	CCC	GAA	CCG	GTG	ACG	GTG	TCG	TGG	AAC	TCA						
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---						
	G	C	L	V	K	D	Y	F	P	E	P	V	T	V	S	W	N	S						
			549			558			567			576			585			594						
	GGC	GCC	CTG	ACC	AGC	GGC	GTG	CAC	ACC	TTC	CCG	GCT	GTC	CTA	CAG	TCC	TCA	GGA						
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---						
	G	A	L	T	S	G	V	H	T	F	P	A	V	L	Q	S	S	G						
			603			612			621			630			639			648						
	CTC	TAC	TCC	CTC	AGC	AGC	GTG	GTG	ACC	GTG	CCC	TCC	AGC	AGC	TTG	GGC	ACC	CAG						
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---						
	L	Y	S	L	S	S	V	V	T	V	P	S	S	S	L	G	T	Q						
			657			666			675			684			693			702						

**Fig. 10(B)**  
(Sheet 1 of 4)

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```

ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA
---
T   Y   I   C   N   V   N   H   K   P   S   N   T   K   V   D   K   K

      711      720      729      738      747      756
GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT
---
V   E   P   K   S   C   D   K   T   H   T   C   P   P   C   P   A   P

      765      774      783      792      801      810
GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC
---
E   L   L   G   G   P   S   V   F   L   F   P   P   K   P   K   D   T

      819      828      837      846      855      864
CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC
---
L   M   I   S   R   T   P   E   V   T   C   V   V   V   D   V   S   H

      873      882      891      900      909      918
GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT
---
E   D   P   E   V   K   F   N   W   Y   V   D   G   V   E   V   H   N

      927      936      945      954      963      972
GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC
---
A   K   T   K   P   R   E   E   Q   Y   N   S   T   Y   R   V   V   S

      981      990      999      1008      1017      1026
GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG
---
V   L   T   V   L   H   Q   D   W   L   N   G   K   E   Y   K   C   K

      1035      1044      1053      1062      1071      1080
GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA
---
V   S   N   K   A   L   P   A   P   I   E   K   T   I   S   K   A   K

      1089      1098      1107      1116      1125      1134
GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG
---
G   Q   P   R   E   P   Q   V   Y   T   L   P   P   S   R   D   E   L

      1143      1152      1161      1170      1179      1188
ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC
---
T   K   N   Q   V   S   L   T   C   L   V   K   G   F   Y   P   S   D

      1197      1206      1215      1224      1233      1242
ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG
---
I   A   V   E   W   E   S   N   G   Q   P   E   N   N   Y   K   T   T

      1251      1260      1269      1278      1287      1296
CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG
---
P   P   V   L   D   S   D   G   S   F   F   L   Y   S   K   L   T   V

      1305      1314      1323      1332      1341      1350
GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG
---
D   K   S   R   W   Q   Q   G   N   V   F   S   C   S   V   M   H   E

```

**Fig. 10(B)**  
(Sheet 2 of 4)

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1359	1368	1377	1386	1395	1404
GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG GGT AAA GGG					
A L H N H Y T Q K S L S L S P G K <u>G</u>					
1413	1422	1431	1440	1449	1458
AGC GGC GGG CTG AAG ATC GCA GCC TTC AAC ATC CAG ACA TTT GGG GAG ACC AAG					
<u>S G G</u> L K I A A F N I Q T F G E T K					
1467	1476	1485	1494	1503	1512
ATG TCC AAT GCC ACC CTC GTC AGC TAC ATT GTG CAG ATC CTG AGC CGC TAC GAC					
M S N A T L V S Y I V Q I L S R Y D					
1521	1530	1539	1548	1557	1566
ATC GCC CTG GTC CAG GAG GTC AGA GAC AGC CAC CTG ACT GCC GTG GGG AAG CTG					
I A L V Q E V R D S H L T A V G K L					
1575	1584	1593	1602	1611	1620
CTG GAC AAC CTC AAT CAG GAC GCA CCA GAC ACC TAT CAC TAC GTG GTC AGT GAG					
L D N L N Q D A P D T Y H Y V V S E					
1629	1638	1647	1656	1665	1674
CCA CTG GGA CGG AAC AGC TAT AAG GAG CGC TAC CTG TTC GTG TAC AGG CCT GAC					
P L G R N S Y K E R Y L F V Y R P D					
1683	1692	1701	1710	1719	1728
CAG GTG TCT GCG GTG GAC AGC TAC TAC TAC GAT GAT GGC TGC GAG CCC TGC GGG					
Q V S A V D S Y Y Y D D G C E P C G					
1737	1746	1755	1764	1773	1782
AAC GAC ACC TTC AAC CGA GAG CCA GCC ATT GTC AGG TTC TTC TCC CGG TTC ACA					
N D T F N R E P A I V R F F S R F T					
1791	1800	1809	1818	1827	1836
GAG GTC AGG GAG TTT GCC ATT GTT CCC CTG CAT GCG GCC CCG GGG GAC GCA GTA					
E V R E F A I V P L H A A P G D A V					
1845	1854	1863	1872	1881	1890
GCC GAG ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT GTC CAA GAG AAA TGG GGC					
A E I D A L Y D V Y L D V Q E K W G					
1899	1908	1917	1926	1935	1944
TTG GAG GAC GTC ATG TTG ATG GGC GAC TTC AAT GCG GGC TGC AGC TAT GTG AGA					
L E D V M L M G D F N A G C S Y V R					
1953	1962	1971	1980	1989	1998
CCC TCC CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC CCC ACC TTC CAG TGG CTG					
P S Q W S S I R L W T S P T F Q W L					
2007	2016	2025	2034	2043	2052
ATC CCC GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG CAC TGT GCC TAT GAC AGG					
I P D S A D T T A T P T H C A Y D R					

**Fig. 10(B)**  
**(Sheet 3 of 4)**

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2061	2070	2079	2088	2097	2106
ATC GTG GTT GCA GGG ATG CTG CTC CGA GGG GCC GTT GTT CCC GAC TCG GCT CTT					
---	---	---	---	---	---
I V V A G M L L R G A V V P D S A L					
2115	2124	2133	2142	2151	2160
CCC TTT AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC CAA CTG GCC CAA GCC ATC					
---	---	---	---	---	---
P F N F Q A A Y G L S D Q L A Q A I					
2169	2178	2187	2196	2205	2214
AGT GAC CAC TAT CCA GTG GAG GTG ATG CTG AAG GGG GGC GGA CCC AAA AAG AAG					
---	---	---	---	---	---
S D H Y P V E V M L K			<u>G G G P K K K</u>		
2223					
CGC AAG GTT TGA 3'					
---					
<u>R K V</u> *					

***Fig. 10(B)***  
***(Sheet 4 of 4)***

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## pAS38

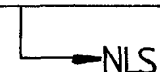
LOCUS PAS38.DNA 2223 bp 2193 bp DNA 14-AUG-1998  
 DEFINITION HUMANISED HMFG1 heavy chain fused to human DNase construct 38  
 DEFINITION Clone 17.12.1 with silent K to K mutation (1398 A > G)+NLS  
 REFERENCE  
 AUTHORS VERHOEYEN ET AL  
 TITLE CONSTRUCTION OF RESHAPED HMFG1 etc  
 JOURNAL IMMUNOL. (1993):78, 364-370  
 COMMENT Human DNase sequence is modified as a result of oligo assembly (mhdnase.dna)  
 COMMENT The fusion was made using overlapping oligos AS81 and AS82  
 FEATURES AA RESIDUE 235 HAS NOT BEEN CHANGED TO KABAT (I.E. V TO A)  
 FEATURES Residue 963 is G > T leading to silent mutation in all clones  
 FEATURES In 17.12.1 residue 1398 is A > G (silent K to K mutation)  
 SITES Note  
 BASE COUNT 510 a 683 c 618 g 412 t  
 ORIGIN ?

```

1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
121 TGCAAGGCTT CTGGCTACAC CTTCAAGTGC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAAC TCA
541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCTTC AGGACTCTAC
601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAG CTACATCTGC
661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
721 GACAAAAC TC ACACATGCCC ACCGTGCCCC GCACCTGAAC TCCTGGGGGG ACCGTCAGTC
781 TTCTCTTCC CCCCCAAACC CAAGGACACC CTCATGATCT CCCGGACCCC TGAGGT CACA
841 TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC
901 GCGGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC
961 CGTGTGGTCA GCGTCCTCAC CGTCCTGCAC CAGGACTGGC TGAATGGCAA GGAGTACAAAG
1021 TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA
1081 GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCCGGGATGA GCTGACCAAG
1141 AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG
1201 TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCTG CTTGGACTCC
1261 GACGGCTCCT TCTTCTCTTA CAGCAAGCTC ACCGTGGACA AGAGCAGGTG GCAGAGGGG
1321 AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC
1381 CTCTCCCTGT CTCCGAAG GG GAGCGGCGGG CTGAAGATCG CAGCCTTCAA CATCCAGACA
1441 TTTGGGGAGA CCAAGATGTC CAATGCCACC CTCGTCAGCT ACATTGTGCA GATCCTGAGC
1501 CGCTACGACA TCGCCCTGGT CCAGGAGGTC AGAGACAGCC ACCTGACTGC CGTGGGGAAG
1561 CTGCTGGACA ACCTCAATCA GGACGCACCA GACACCTATC ACTACGTGGT CAGTGAGCCA
1621 CTGGGACGGA ACAGCTATAA GGAGCGCTAC CTGTTCTGTG ACAGGCCTGA CCAGGTGTCT
1681 GCGGTGGACA GCTACTACTA CGATGATGGC TGCGAGCCCT GCGGGAACGA CACCTTCAAC
1741 CGAGAGCCAG CCATTGTCAG GTTCTTCTCC CGGTTACAG AGGTCAGGGA GTTTGCCATT
1801 GTTCCCCTGC ATGCGGCCCC GGGGGACGCA GTAGCCGAGA TCGACGCTCT CTATGACGTC
1861 TACCTGGATG TCCAAGAGAA ATGGGGCTTG GAGGACGTCA TGTTGATGGG CGACTTCAAT
1921 GCGGGCTGCA GCTATGTGAG ACCCTCCCAG TGGTCATCCA TCCGCTGTG GACAAGCCCC
1981 ACCTTCCAGT GGCTGATCCC CGACAGCGCT GACACCACAG CTACACCCAC GCACTGTGCC
2041 TATGACAGGA TCGTGGTTGC AGGGATGCTG CTCCGAGGGG CCGTTGTTCC CGACTCGGCT
2101 CTTCCCTTTA ACTTCCAGGC TGCCTATGGC CTGAGTGACC AACTGGCCCA AGCCATCAGT
2161 GACCACTATC CAGTGGAGGT GATGCTGAAG GGGGGCGGAC CCAAAAAGAA GCGCAAGGTT
2221 TGA

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NLS

**Fig. 11(A)**

5	ATG	GGA	TGG	AGC	TGT	ATC	ATC	CTC	TTC	TTG	GTA	GCA	ACA	GCT	ACA	GGT	GTC	CAC	54
	M	G	W	S	C	I	I	L	F	L	V	A	T	A	T	G	V	H	
			63			72			81			90			99			108	
	TCC	CAG	GTG	CAG	CTG	GTG	CAG	TCT	GGG	GCA	GAG	GTG	AAA	AAG	CCT	GGG	GCC	TCA	
	S	Q	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S	
			117			126			135			144			153			162	
	GTG	AAG	GTG	TCC	TGC	AAG	GCT	TCT	GGC	TAC	ACC	TTC	AGT	GCC	TAC	TGG	ATA	GAG	
	V	K	V	S	C	K	A	S	G	Y	T	F	S	A	Y	W	I	E	
			171			180			189			198			207			216	
	TGG	GTG	CGC	CAG	GCT	CCA	GGA	AAG	GGC	CTC	GAG	TGG	GTC	GGA	GAG	ATT	TTA	CCT	
	W	V	R	Q	A	P	G	K	G	L	E	W	V	G	E	I	L	P	
			225			234			243			252			261			270	
	GGA	AGT	AAT	AAT	TCT	AGA	TAC	AAT	GAG	AAG	TTC	AAG	GGC	CGA	GTG	ACA	GTC	ACT	
	G	S	N	N	S	R	Y	N	E	K	F	K	G	R	V	T	V	T	
			279			288			297			306			315			324	
	AGA	GAC	ACA	TCC	ACA	AAC	ACA	GCC	TAC	ATG	GAG	CTC	AGC	AGC	CTG	AGG	TCT	GAG	
	R	D	T	S	T	N	T	A	Y	M	E	L	S	S	L	R	S	E	
			333			342			351			360			369			378	
	GAC	ACA	GCC	GTC	TAT	TAC	TGT	GCA	AGA	TCC	TAC	GAC	TTT	GCC	TGG	TTT	GCT	TAC	
	D	T	A	V	Y	Y	C	A	R	S	Y	D	F	A	W	F	A	Y	
			387			396			405			414			423			432	
	TGG	GGC	CAA	GGG	ACT	CTG	GTC	ACA	GTC	TCC	TCA	GCC	TCC	ACC	AAG	GGC	CCA	TGG	
	W	G	Q	G	T	L	V	T	V	S	S	A	S	T	K	G	P	S	
			441			450			459			468			477			486	
	GTC	TTC	CCC	CTG	GCA	CCC	TCC	TCC	AAG	AGC	ACC	TCT	GGG	GGC	ACA	GCG	GCC	CTG	
	V	F	P	L	A	P	S	S	K	S	T	S	G	G	T	A	A	L	
			495			504			513			522			531			540	
	GGC	TGC	CTG	GTC	AAG	GAC	TAC	TTC	CCC	GAA	CCG	GTG	ACG	GTG	TCG	TGG	AAC	TCA	
	G	C	L	V	K	D	Y	F	P	E	P	V	T	V	S	W	N	S	
			549			558			567			576			585			594	
	GGC	GCC	CTG	ACC	AGC	GGC	GTG	CAC	ACC	TTC	CCG	GCT	GTC	CTA	CAG	TCC	TCA	GGA	
	G	A	L	T	S	G	V	H	T	F	P	A	V	L	Q	S	S	G	
			603			612			621			630			639			648	
	CTC	TAC	TCC	CTC	AGC	AGC	GTG	GTG	ACC	GTG	CCC	TCC	AGC	AGC	TTG	GGC	ACC	CAG	
	L	Y	S	L	S	S	V	V	T	V	P	S	S	S	L	G	T	Q	
			657			666			675			684							

**Fig. 11(B)**  
**(Sheet 1 of 4)**

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ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA
---
T   Y   I   C   N   V   N   H   K   P   S   N   T   K   V   D   K   K

      711      720      729      738      747      756
GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT
---
V   E   P   K   S   C   D   K   T   H   T   C   P   P   C   P   A   P

      765      774      783      792      801      810
GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC
---
E   L   L   G   G   P   S   V   F   L   F   P   P   K   P   K   D   T

      819      828      837      846      855      864
CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC
---
L   M   I   S   R   T   P   E   V   T   C   V   V   V   D   V   S   H

      873      882      891      900      909      918
GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT
---
E   D   P   E   V   K   F   N   W   Y   V   D   G   V   E   V   H   N

      927      936      945      954      963      972
GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC
---
A   K   T   K   P   R   E   E   Q   Y   N   S   T   Y   R   V   V   S

      981      990      999      1008      1017      1026
GTC CTC ACC GTC CTG CAC GAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG
---
V   L   T   V   L   H   Q   D   W   L   N   G   K   E   Y   K   C   K

      1035      1044      1053      1062      1071      1080
GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA
---
V   S   N   K   A   L   P   A   P   I   E   K   T   I   S   K   A   K

      1089      1098      1107      1116      1125      1134
GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG
---
G   Q   P   R   E   P   Q   V   Y   T   L   P   P   S   R   D   E   L

      1143      1152      1161      1170      1179      1188
ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC
---
T   K   N   Q   V   S   L   T   C   L   V   K   G   F   Y   P   S   D

      1197      1206      1215      1224      1233      1242
ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG
---
I   A   V   E   W   E   S   N   G   Q   P   E   N   N   Y   K   T   T

      1251      1260      1269      1278      1287      1296
CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG
---
P   P   V   L   D   S   D   G   S   F   F   L   Y   S   K   L   T   V

      1305      1314      1323      1332      1341      1350
GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG
---
D   K   S   R   W   Q   Q   G   N   V   F   S   C   S   V   M   H   E

```

**Fig. 11(B)**  
**(Sheet 2 of 4)**

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1359	1368	1377	1386	1395	1404
GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG AAG GGG AGC					
A L H N H Y T Q K S L S L S P K <u>G S</u>					
1413	1422	1431	1440	1449	1458
GGC GGG CTG AAG ATC GCA GCC TTC AAC ATC CAG ACA TTT GGG GAG ACC AAG ATG					
<u>G G</u> L K I A A F N I Q T F G E T K M					
1467	1476	1485	1494	1503	1512
TCC AAT GCC ACC CTC GTC AGC TAC ATT GTG CAG ATC CTG AGC CGC TAC GAC ATC					
S N A T L V S Y I V Q I L S R Y D I					
1521	1530	1539	1548	1557	1566
GCC CTG GTC CAG GAG GTC AGA GAC AGC CAC CTG ACT GCC GTG GGG AAG CTG CTG					
A L V Q E V R D S H L T A V G K L L					
1575	1584	1593	1602	1611	1620
GAC AAC CTC AAT CAG GAC GCA CCA GAC ACC TAT CAC TAC GTG GTC AGT GAG CCA					
D N L N Q D A P D T Y H Y V V S E P					
1629	1638	1647	1656	1665	1674
CTG GGA CGG AAC AGC TAT AAG GAG CGC TAC CTG TTC GTG TAC AGG CCT GAC CAG					
L G R N S Y K E R Y L F V Y R P D Q					
1683	1692	1701	1710	1719	1728
GTG TCT GCG GTG GAC AGC TAC TAC TAC GAT GAT GGC TGC GAG CCC TGC GGG AAC					
V S A V D S Y Y Y D D G C E P C G N					
1737	1746	1755	1764	1773	1782
GAC ACC TTC AAC CGA GAG CCA GCC ATT GTC AGG TTC TTC TCC CGG TTC ACA GAG					
D T F N R E P A I V R F F S R F T E					
1791	1800	1809	1818	1827	1836
GTC AGG GAG TTT GCC ATT GTT CCC CTG CAT GCG GCC CCG GGG GAC GCA GTA GCC					
V R E F A I V P L H A A P G D A V A					
1845	1854	1863	1872	1881	1890
GAG ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT GTC CAA GAG AAA TGG GGC TTG					
E I D A L Y D V Y L D V Q E K W G L					
1899	1908	1917	1926	1935	1944
GAG GAC GTC ATG TTG ATG GGC GAC TTC AAT GCG GGC TGC AGC TAT GTG AGA CCC					
E D V M L M G D F N A G C S Y V R P					
1953	1962	1971	1980	1989	1998
TCC CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC CCC ACC TTC CAG TGG CTG ATC					
S Q W S I R L W T S P T F Q W L I					
2007	2016	2025	2034	2043	2052
CCC GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG CAC TGT GCC TAT GAC AGG ATC					
P D S A D T T A T P T H C A Y D R I					

**Fig. 11(C)**  
**(Sheet 3 of 4)**

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2061	2070	2079	2088	2097	2106
GTG GTT GCA GGG ATG CTG CTC CGA GGG GCC GTT GTT CCC GAC TCG GCT CTT CCC					
---	---	---	---	---	---
V V A G M L L R G A V V P D S A L P					
2115	2124	2133	2142	2151	2160
TTT AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC CAA CTG GCC CAA GCC ATC AGT					
---	---	---	---	---	---
F N F Q A A Y G L S D Q L A Q A I S					
2169	2178	2187	2196	2205	2214
GAC CAC TAT CCA GTG GAG GTG ATG CTG AAG GGG GGC GGA CCC AAA AAG AAG CGC					
---	---	---	---	---	---
D H Y P V E V M L K			<u>G G G P K K K R</u>		
2223					
AAG GTT TGA 3'					
---					
K V *					

***Fig. 11(D)***  
***(Sheet 4 of 4)***

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## pAS39

LOCUS PAS39.DNA 2220 bp 2190 bp DNA 14-AUG-1998

DEFINITION HUMANISED HMFG1 heavy chain fused to human DNase - construct 39

DEFINITION Clone 18.24.1 with residue 1392 T > C +NLS

REFERENCE

AUTHORS VERHOEYEN ET AL

TITLE CONSTRUCTION OF RESHAPED HMFG1 etc

JOURNAL IMMUNOL. (1993):78, 364-370

COMMENT Human DNase sequence is modified as a result of oligo assembly (mhdnase.dna)

COMMENT The fusion was made using overlapping oligos AS83 and AS84

FEATURES AA RESIDUE 235 HAS NOT BEEN CHANGED TO KABAT (I.E. V TO A)

FEATURES Residue 963 is G > T leading to silent mutation in all clones

FEATURES Residue 1392 T > C silent S to S mutation

SITES Note

BASE COUNT 508 a 684 c 617 g 411 t

ORIGIN ?

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1  ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
61  GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
121 TGCAAGGCTT CTGGCTACAC CTTCAAGTGC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAAGTCA
541 GCGGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
721 GACAAAATC ACACATGCCC ACCGTGCCCC GCACCTGAAC TCCTGGGGGG ACCGTCAGTC
781 TTCCTCTTCC CCCCAAAACC CAAGGACACC CTCATGATCT CCCGGACCCC TGAGGTCACA
841 TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC
901 GGCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC
961 CGTCTGGTCA GCGTCCTCAC CGTCCTGCAC CAGGACTGGC TGAATGGCAA GGAGTACAAG
1021 TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA
1081 GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCCGGGATGA GCTGACCAAG
1141 AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG
1201 TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT GCTGGACTCC
1261 GACGGCTCCT TCTTCCTCTA CAGCAAGCTC ACCGTGGACA AGAGCAGGTG GCAGCAGGGG
1321 AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC
1381 CTCTCCCTGT CcCCGGGGAG CGGCGGGCTG AAGATCGCAG CCTTCAACAT CCAGACATTT
1441 GGGGAGACCA AGATGTCCAA TGCCACCCTC GTCAGTACA TTGTGCAGAT CCTGAGCCGC
1501 TACGACATCG CCCTGGTCCA GGAGGTCAGA GACAGCCACC TGACTGCCGT GGGGAAGCTG
1561 CTGGACAACC TCAATCAGGA CGCACCAGAC ACCTATCACT ACGTGGTCAG TGAGCCACTG
1621 GGACGGAACA GCTATAAGGA GCGCTACCTG TTCGTGTACA GGCCTGACCA GGTGTCTGCG
1681 GTGGACAGCT ACTACTACGA TGATGGCTGC GAGCCCTGCG GGAACGACAC CTTCAACCGA
1741 GAGCCAGCCA TTGTCAGGTT CTTCTCCCGG TTCACAGAGG TCAGGGAGTT TGCCATTGTT
1801 CCCCTGCATG CGGCCCCGGG GGACGCAGTA GCCGAGATCG ACGCTCTCTA TGACGTCTAC
1861 CTGGATGTCC AAGAGAAATG GGGCTTGGAG GACGTCATGT TGATGGGCGA CTTCAATGCG
1921 GGCTGCAGCT ATGTGAGACC CTCCCAGTGG TCATCCATCC GCCTGTGGAC AAGCCCCACC
1981 TTCCAGTGGC TGATCCCCGA CAGCGCTGAC ACCACAGCTA CACCCACGCA CTGTGCCTAT
2041 GACAGGATCG TGGTTGCAGG GATGCTGCTC CGAGGGGCGG TTGTTCCCGA CTCGGCTCTT
2101 CCCTTTAACT TCCAGGCTGC CTATGGCCTG AGTGACCAAC TGGCCCAAGC CATCAGTGAC
2161 CACTATCCAG TGGAGGTGAT GCTGAAGGGG GGCGGACCCA AAAAGAAGCG CAAGGTTTGA

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**Fig. 12(A)**

			9			18			27			36			45			54
5'	ATG	GGA	TGG	AGC	TGT	ATC	ATC	CTC	TTC	TTG	GTA	GCA	ACA	GCT	ACA	GGT	GTC	CAC
	M	G	W	S	C	I	I	L	F	L	V	A	T	A	T	G	V	H
			63			72			81			90			99			108
	TCC	CAG	GTG	CAG	CTG	GTG	CAG	TCT	GGG	GCA	GAG	GTG	AAA	AAG	CCT	GGG	GCC	TCA
	S	Q	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S
			117			126			135			144			153			162
	GTG	AAG	GTG	TCC	TGC	AAG	GCT	TCT	GGC	TAC	ACC	TTC	AGT	GCC	TAC	TGG	ATA	GAG
	V	K	V	S	C	K	A	S	G	Y	T	F	S	A	Y	W	I	E
			171			180			189			198			207			216
	TGG	GTG	CGC	CAG	GCT	CCA	GGA	AAG	GGC	CTC	GAG	TGG	GTC	GGA	GAG	ATT	TTA	CCT
	W	V	R	Q	A	P	G	K	G	L	E	W	V	G	E	I	L	P
			225			234			243			252			261			270
	GGA	AGT	AAT	AAT	TCT	AGA	TAC	AAT	GAG	AAG	TTC	AAG	GGC	CGA	GTG	ACA	GTC	ACT
	G	S	N	N	S	R	Y	N	E	K	F	K	G	R	V	T	V	T
			279			288			297			306			315			324
	AGA	GAC	ACA	TCC	ACA	AAC	ACA	GCC	TAC	ATG	GAG	CTC	AGC	AGC	CTG	AGG	TCT	GAG
	R	D	T	S	T	N	T	A	Y	M	E	L	S	S	L	R	S	E
			333			342			351			360			369			378
	GAC	ACA	GCC	GTC	TAT	TAC	TGT	GCA	AGA	TCC	TAC	GAC	TTT	GCC	TGG	TTT	GCT	TAC
	D	T	A	V	Y	Y	C	A	R	S	Y	D	F	A	W	F	A	Y
			387			396			405			414			423			432
	TGG	GGC	CAA	GGG	ACT	CTG	GTC	ACA	GTC	TCC	TCA	GCC	TCC	ACC	AAG	GGC	CCA	TCG
	W	G	Q	G	T	L	V	T	V	S	S	A	S	T	K	G	P	S
			441			450			459			468			477			486
	GTC	TTC	CCC	CTG	GCA	CCC	TCC	TCC	AAG	AGC	ACC	TCT	GGG	GGC	ACA	GCG	GCC	CTG
	V	F	P	L	A	P	S	S	K	S	T	S	G	G	T	A	A	L
			495			504			513			522			531			540
	GGC	TGC	CTG	GTC	AAG	GAC	TAC	TTC	CCC	GAA	CCG	GTG	ACG	GTG	TCG	TGG	AAC	TCA
	G	C	L	V	K	D	Y	F	P	E	P	V	T	V	S	W	N	S
			549			558			567			576			585			594
	GGC	GCC	CTG	ACC	AGC	GGC	GTG	CAC	ACC	TTC	CCG	GCT	GTC	CTA	CAG	TCC	TCA	GGA
	G	A	L	T	S	G	V	H	T	F	P	A	V	L	Q	S	S	G
			603			612			621			630			639			648
	CTC	TAC	TCC	CTC	AGC	AGC	GTG	GTG	ACC	GTG	CCC	TCC	AGC	AGC	TTG	GGC	ACC	CAG
	L	Y	S	L	S	S	V	V	T	V	P	S	S	S	L	G	T	Q
			657			666			675			684			693			702

**Fig. 12(B)**  
(Sheet 1 of 4)



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ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA
---
T   Y   I   C   N   V   N   H   K   P   S   N   T   K   V   D   K   K

      711      720      729      738      747      756
GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT
---
V   E   P   K   S   C   D   K   T   H   T   C   P   P   C   P   A   P

      765      774      783      792      801      810
GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC
---
E   L   L   G   G   P   S   V   F   L   F   P   P   K   P   K   D   T

      819      828      837      846      855      864
CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC
---
L   M   I   S   R   T   P   E   V   T   C   V   V   V   D   V   S   H

      873      882      891      900      909      918
GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT
---
E   D   P   E   V   K   F   N   W   Y   V   D   G   V   E   V   H   N

      927      936      945      954      963      972
GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC
---
A   K   T   K   P   R   E   E   Q   Y   N   S   T   Y   R   V   V   S

      981      990      999      1008      1017      1026
GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG
---
V   L   T   V   L   H   Q   D   W   L   N   G   K   E   Y   K   C   K

      1035      1044      1053      1062      1071      1080
GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA
---
V   S   N   K   A   L   P   A   P   I   E   K   T   I   S   K   A   K

      1089      1098      1107      1116      1125      1134
GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG
---
G   Q   P   R   E   P   Q   V   Y   T   L   P   P   S   R   D   E   L

      1143      1152      1161      1170      1179      1188
ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC
---
T   K   N   Q   V   S   L   T   C   L   V   K   G   F   Y   P   S   D

      1197      1206      1215      1224      1233      1242
ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG
---
I   A   V   E   W   E   S   N   G   Q   P   E   N   N   Y   K   T   T

      1251      1260      1269      1278      1287      1296
CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG
---
P   P   V   L   D   S   D   G   S   F   F   L   Y   S   K   L   T   V

      1305      1314      1323      1332      1341      1350
GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG
---
D   K   S   R   W   Q   Q   G   N   V   F   S   C   S   V   M   H   E

```

**Fig. 12(B)**  
**(Sheet 2 of 4)**

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1359	1368	1377	1386	1395	1404
GCT CTG CAC AAC	CAC TAC ACG	CAG AAG AGC	CTC TCC CTG	TCC CCG GGG	AGC GGC
---	---	---	---	---	---
A L H N	H Y T	Q K S	L S L	S P	<u>G S G</u>
1413	1422	1431	1440	1449	1458
GGG CTG AAG ATC	GCA GCC TTC	AAC ATC CAG	ACA TTT GGG	GAG ACC AAG	ATG TCC
---	---	---	---	---	---
<u>G</u> L K I	A A F	N I Q	T F G	E T K	M S
1467	1476	1485	1494	1503	1512
AAT GCC ACC CTC	GTC AGC TAC	ATT GTG CAG	ATC CTG AGC	CGC TAC GAC	ATC GCC
---	---	---	---	---	---
N A T L	V S Y	I V Q	I L S	R Y D	I A
1521	1530	1539	1548	1557	1566
CTG GTC CAG GAG	GTC AGA GAC	AGC CAC CTG	ACT GCC GTG	GGG AAG CTG	CTG GAC
---	---	---	---	---	---
L V Q E	V R D	S H L	T A V	G K L	L D
1575	1584	1593	1602	1611	1620
AAC CTC AAT CAG	GAC GCA CCA	GAC ACC TAT	CAC TAC GTG	GTC AGT GAG	CCA CTG
---	---	---	---	---	---
N L N Q	D A P	D T Y	H Y V	V S E	P L
1629	1638	1647	1656	1665	1674
GGA CGG AAC AGC	TAT AAG GAG	CGC TAC CTG	TTC GTG TAC	AGG CCT GAC	CAG GTG
---	---	---	---	---	---
G R N S	Y K E	R Y L	F V Y	R P D	Q V
1683	1692	1701	1710	1719	1728
TCT GCG GTG GAC	AGC TAC TAC	TAC GAT GAT	GGC TGC GAG	CCC TGC GGG	AAC GAC
---	---	---	---	---	---
S A V D	S Y Y	Y D D	G C E	P C G	N D
1737	1746	1755	1764	1773	1782
ACC TTC AAC CGA	GAG CCA GCC	ATT GTC AGG	TTC TTC TCC	CGG TTC ACA	GAG GTC
---	---	---	---	---	---
T F N R	E P A	I V R	F F S	R F T	E V
1791	1800	1809	1818	1827	1836
AGG GAG TTT GCC	ATT GTT CCC	CTG CAT GCG	GCC CCG GGG	GAC GCA GTA	GCC GAG
---	---	---	---	---	---
R E F A	I V P	L H A	A P G	D A V	A E
1845	1854	1863	1872	1881	1890
ATC GAC GCT CTC	TAT GAC GTC	TAC CTG GAT	GTC CAA GAG	AAA TGG GGC	TTG GAG
---	---	---	---	---	---
I D A L	Y D V	Y L D	V Q E	K W G	L E
1899	1908	1917	1926	1935	1944
GAC GTC ATG TTG	ATG GGC GAC	TTC AAT GCG	GGC TGC AGC	TAT GTG AGA	CCC TCC
---	---	---	---	---	---
D V M L	M G D	F N A	G C S	Y V R	P S
1953	1962	1971	1980	1989	1998
CAG TGG TCA TCC	ATC CGC CTG	TGG ACA AGC	CCC ACC TTC	CAG TGG CTG	ATC CCC
---	---	---	---	---	---
Q W S S	I R L	W T S	P T F	Q W L	I P
2007	2016	2025	2034	2043	2052
GAC AGC GCT GAC	ACC ACA GCT	ACA CCC ACG	CAC TGT GCC	TAT GAC AGG	ATC GTG
---	---	---	---	---	---
D S A D	T T A	T P T	H C A	Y D R	I V

**Fig. 12(B)**  
**(Sheet 3 of 4)**

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2061				2070				2079				2088				2097				2106	
GTT	GCA	GGG	ATG	CTG	CTC	CGA	GGG	GCC	GTT	GTT	CCC	GAC	TCG	GCT	CTT	CCC	TTT				
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---				
V	A	G	M	L	L	R	G	A	V	V	P	D	S	A	L	P	F				
2115				2124				2133				2142				2151				2160	
AAC	TTC	CAG	GCT	GCC	TAT	GGC	CTG	AGT	GAC	CAA	CTG	GCC	CAA	GCC	ATC	AGT	GAC				
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---				
N	F	Q	A	A	Y	G	L	S	D	Q	L	A	Q	A	I	S	D				
2169				2178				2187				2196				2205				2214	
CAC	TAT	CCA	GTG	GAG	GTG	ATG	CTG	AAG	GGG	GGC	GGA	CCC	AAA	AAG	AAG	CGC	AAG				
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---				
H	Y	P	V	E	V	M	L	K	G	G	G	P	K	K	K	R	K				

**Fig. 12(B)**  
**(Sheet 4 of 4)**

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## pAS101

LOCUS PAS101.DNA 1548 bp mRNA PRI 06-MAR-1995  
 DEFINITION Humanised HMFG1 Fab'2 fused to human DNase I (pAS101)  
 ACCESSION  
 NID  
 KEYWORDS DNase I.  
 SOURCE DNase I sequence is from assembled oligos (thus modified c/f MHDNASE1.dna)  
 ORGANISM Homo sapiens  
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata; Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.  
 AUTHORS Shak,S., Capon,D.J., Hellmiss,R., Marsters,S.A. and Baker,C.L.  
 TITLE Recombinant human DNase I reduces the viscosity of cystic fibrosis sputum  
 JOURNAL Proc. Natl. Acad. Sci. U.S.A. 87 (23), 9188-9192 (1990)  
 MEDLINE 91067672  
 BASE COUNT 343 a 467 c 430 g 308 t  
 ORIGIN

```

1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
121 TGCAAGGCTT CTGGCTACAC CTTCAAGTGC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
481 GCCCTGGGCT GCCTGGTCAA GGAATACTTC CCCGAACCGG TGACGGTGTC GTGGAAGTCA
541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCTCT AGGACTCTAC
601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
721 GACAAAATCT ACACATGCCC ACCGTGCCCC GCACCTGAAG GCGGGCTGAA GATCGCAGCC
781 TTCAACATCC AGACATTGTT GGAGACCAAG ATGTCCAATG CCACCCTCGT CAGCTACATT
841 GTGCAGATCC TGAGCCGCTA CGACATCGCC CTGGTCCAGG AGGTGAGAGA CAGCCACCTG
901 ACTGCCGTGG GGAAGCTGCT GGACAACCTC AATCAGGACG ACCAGACAC CTATCACTAC
961 GTGGTCAGTG AGCCACTGGG ACGGAACAGC TATAAGGAGC GCTACCTGTT CGTGTACAGG
1021 CCTGACCAGG TGTCTGCGGT GGACAGCTAC TACTACGATG ATGGCTGCGA GCCCTGCGGG
1081 AACGACACCT TCAACCGAGA GCCAGCCATT GTCAGGTTCT TCTCCCGGTT CACAGAGGTC
1141 AGGGAGTTTG CCATTGTTCC CCTGCATGCG GCCCGGGGGG ACGCAGTAGC CGAGATCGAC
1201 GCTCTCTATG ACGTCTACCT GGATGTCCAA GAGAAATGGG GCTTGGAGGA CGTCATGTTG
1261 ATGGGCGACT TCAATGCGGG CTGCAGCTAT GTGAGACCCT CCCAGTGGTC ATCCATCCGC
1321 CTGTGGACAA GCCCCACCTT CCAGTGGCTG ATCCCCGACA GCGCTGACAC CACAGCTACA
1381 CCGACGCACT GTGCCTATGA CAGGATCGTG GTTGCAAGGA TGCTGCTCCG AGGGGCCGTT
1441 GTTCCCGACT CGGCTCTTCC CTTTAACTTC CAGGCTGCCT ATGGCCTGAG TGACCAACTG
1501 GCCCAAGCCA TCAGTGACCA CTATCCAGTG GAGGTGATGC TGAAGTGA

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//

**Fig. 13(A)**

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LOCUS FDDNASE101 1548 BP SS-DNA SYN 25-AUG-2000  
 DEFINITION -  
 ACCESSION -  
 KEYWORDS -  
 SOURCE -  
 FEATURES Location/Qualifiers  
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   frag 721..780  
     /note="1 to 60 of 101/105linker"  
   frag join(721..>735,<736..>759,<760..>780)  
     /note="1 to 80 of 102linker [Split]"  
 BASE COUNT 343 A 465 C 431 G 309 T 0 OTHER  
 ORIGIN -  
   1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG  
   61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC  
  121 TGCAAGGCTT CTGGCTACAC CTTCAAGTGC TACTGGATAG AGTGGGTGCG CCAGGCTCCA  
  181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT  
  241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG  
  301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC  
  361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC  
  421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG  
  481 GCCCTGGGCT GCCTGGTCAA GGAATACTTC CCCGAACCGG TGACGGTGTC GTGGAATCA  
  541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC  
  601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC  
  661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT  
  721 GACAAAATC ACACATGTCC ACCGTGTCCA GCACCAGAGG GCGGGCTGAA GATCGCAGCC  
  781 TTCAACATCC AGACATTGAG GGAGACCAAG ATGTCCAATG CCACCCTCGT CAGCTACATT  
  841 GTGCAGATCC TGAGCCGCTA CGACATCGCC CTGGTCCAGG AGGTGAGAGA CAGCCACCTG  
  901 ACTGCCGTGG GGAAGCTGCT GGACAACCTC AATCAGGACG CACCAGACAC CTATCACTAC  
  961 GTGGTCAGTG AGCCACTGGG ACGGAACAGC TATAAGGAGC GCTACCTGTT CGTGTACAGG  
 1021 CCTGACCAGG TGTCTGCGGT GGACAGCTAC TACTACGATG ATGGCTGCGA GCCCTGCGGG  
 1081 AACGACACCT TCAACCGAGA GCCAGCCATT GTCAGGTTCT TCTCCCGGTT CACAGAGGTC  
 1141 AGGGAGTTTG CCATTGTTCC CCTGCATGCG GCCCCGGGGG ACGCAGTAGC CGAGATCGAC  
 1201 GCTCTCTATG ACGTCTACCT GGATGTCCAA GAGAAATGGG GCTTGGAGGA CGTCATGTTG  
 1261 ATGGGCGACT TCAATGCGGG CTGCAGCTAT GTGAGACCCT CCCAGTGGTC ATCCATCCGC  
 1321 CTGTGGACAA GCCCCACCTT CCAGTGGCTG ATCCCCGACA GCGCTGACAC CACAGCTACA  
 1381 CCCACGCACT GTGCCTATGA CAGGATCGTG GTTGCAAGGA TGCTGCTCCG AGGGGCGGTT  
 1441 GTTCCCGACT CGGCTCTTCC CTTTAACTTC CAGGCTGCCT ATGGCCTGAG TGACCAACTG  
 1501 GCCAAGCCA TCAGTGACCA CTATCCAGTG GAGGTGATGC TGAAGTGA

//

*Fig. 13(B)*

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LOCUS FDDNASE101 1557 BP SS-DNA SYN 29-AUG-2000  
 DEFINITION -  
 ACCESSION -  
 KEYWORDS -  
 SOURCE -  
 FEATURES Location/Qualifiers  
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     /note="1 to 1548 of FdDNase101correct"  
   frag join(10..>729,<790..1557)  
     /note="1 to 1548 of PAS101.dna [Split]"  
   frag 730..789  
     /note="1 to 60 of 101/105linker"  
   frag join(730..>744,<745..>768,<769..>789)  
     /note="1 to 80 of 102linker [Split]"  
 BASE COUNT 344 A 471 C 433 G 309 T 0 OTHER  
 ORIGIN -  
   1 GCGGCCACCA TGGGATGGAG CTGTATCATC CTCTTCTTGG TAGCAACAGC TACAGGTGTC  
   61 CACTCCCAGG TGCAGCTGGT GCAGTCTGGG GCAGAGGTGA AAAAGCCTGG GGCCTCAGTG  
  121 AAGGTGTCCT GCAAGGCTTC TGGCTACACC TTCAGTGCCT ACTGGATAGA GTGGGTGCGC  
  181 CAGGCTCCAG GAAAGGGCCT CGAGTGGGTC GGAGAGATT TACCTGGAAG TAATAATTCT  
  241 AGATACAATG AGAAGTTCAA GGGCCGAGTG ACAGTCACTA GAGACACATC CACAAACACA  
  301 GCCTACATGG AGCTCAGCAG CCTGAGGTCT GAGGACACAG CCGTCTATTA CTGTGCAAGA  
  361 TCCTACGACT TTGCCTGGTT TGCTTACTGG GGCCAAGGGA CTCTGGTCAC AGTCTCCTCA  
  421 GCCTCCACCA AGGGCCCATC GGTCTTCCCC CTGGCACCCCT CCTCCAAGAG CACCTCTGGG  
  481 GGCACAGCGG CCCTGGGCTG CTTGGTCAAG GACTACTTCC CCGAACCAGG GACGGTGTCTG  
  541 TGGAATCAG GCGCCCTGAC CAGCGGCGTG CACACCTTCC CGGCTGTCCT ACAGTCTCTCA  
  601 GGA CTCTACT CCCTCAGCAG CGTGGTGACC GTGCCCTCCA GCAGCTTGGG CACCCAGACC  
  661 TACATCTGCA ACGTGAATCA CAAGCCCAGC AACACCAAGG TGGACAAGAA AGTTGAGCCC  
  721 AAATCTTGTG ACAAACCTCA CACATGTCCA CCGTGTCCAG CACCAGAGGG CGGGCTGAAG  
  781 ATCGCAGCCT TCAACATCCA GACATTTGGG GAGACCAAGA TGTCCAATGC CACCCTCGTC  
  841 AGCTACATTG TGCAGATCCT GAGCCGCTAC GACATCGCCC TGGTCCAGGA GGTCAGAGAC  
  901 AGCCACCTGA CTGCCGTGGG GAAGCTGCTG GACAACCTCA ATCAGGACGC ACCAGACACC  
  961 TATCACTACG TGGTCAGTGA GCCACTGGGA CGGAACAGCT ATAAGGAGCG CTACCTGTTC  
 1021 GTGTACAGGC CTGACCAGGT GTCTGCGGTG GACAGCTACT ACTACGATGA TGGCTGCGAG  
 1081 CCCTGCGGGA ACGACACCTT CAACCGAGAG CCAGCCATTG TCAGGTTCTT CTCCCGGTTT  
 1141 ACAGAGGTCA GGGAGTTTGC CATTGTTCCC CTGCATGCGG CCCCAGGGGA CGCAGTAGCC  
 1201 GAGATCGACG CTCTCTATGA CGTCTACCTG GATGTCCAAG AGAAATGGGG CTTGGAGGAC  
 1261 GTCATGTTGA TGGGCGACTT CAATGCGGGC TGCAGCTATG TGAGACCCTC CCAGTGGTCA  
 1321 TCCATCCGCC TGTGGACAAG CCCACCTTC CAGTGGCTGA TCCCCGACAG CGCTGACACC  
 1381 ACAGCTACAC CCACGCACTG TGCCTATGAC AGGATCGTGG TTGCAGGGAT GCTGCTCCGA  
 1441 GGGGCCGTTG TTCCCGACTC GGCTCTTCCC TTTAACTTCC AGGCTGCCTA TGGCCTGAGT  
 1501 GACCAACTGG CCCAAGCCAT CAGTGACCAC TATCCAGTGG AGGTGATGCT GAAGTGA

//

*Fig. 13(C)*

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```

      9      18      27      36      45      54
5' ATG GGA TGG AGC TGT ATC ATC CTC TTC TTG GTA GCA ACA GCT ACA GGT GTC CAC
   ---
   M  G  W  S  C  I  I  L  F  L  V  A  T  A  T  G  V  H

      63      72      81      90      99      108
TCC CAG GTG CAG CTG GTG CAG TCT GGG GCA GAG GTG AAA AAG CCT GGG GCC TCA
   ---
   S  Q  V  Q  L  V  Q  S  G  A  E  V  K  K  P  G  A  S

      117      126      135      144      153      162
GTG AAG GTG TCC TGC AAG GCT TCT GGC TAC ACC TTC AGT GCC TAC TGG ATA GAG
   ---
   V  K  V  S  C  K  A  S  G  Y  T  F  S  A  Y  W  I  E

      171      180      189      198      207      216
TGG GTG CGC CAG GCT CCA GGA AAG GGC CTC GAG TGG GTC GGA GAG ATT TTA CCT
   ---
   W  V  R  Q  A  P  G  K  G  L  E  W  V  G  E  I  L  P

      225      234      243      252      261      270
GGA AGT AAT AAT TCT AGA TAC AAT GAG AAG TTC AAG GGC CGA GTG ACA GTC ACT
   ---
   G  S  N  N  S  R  Y  N  E  K  F  K  G  R  V  T  V  T

      279      288      297      306      315      324
AGA GAC ACA TCC ACA AAC ACA GCC TAC ATG GAG CTC AGC AGC CTG AGG TCT GAG
   ---
   R  D  T  S  T  N  T  A  Y  M  E  L  S  S  L  R  S  E

      333      342      351      360      369      378
GAC ACA GCC GTC TAT TAC TGT GCA AGA TCC TAC GAC TTT GCC TGG TTT GCT TAC
   ---
   D  T  A  V  Y  Y  C  A  R  S  Y  D  F  A  W  F  A  Y

      387      396      405      414      423      432
TGG GGC CAA GGG ACT CTG GTC ACA GTC TCC TCA GCC TCC ACC AAG GGC CCA TCG
   ---
   W  G  Q  G  T  L  V  T  V  S  S  A  S  T  K  G  P  S

      441      450      459      468      477      486
GTC TTC CCC CTG GCA CCC TCC TCC AAG AGC ACC TCT GGG GGC ACA GCG GCC CTG
   ---
   V  F  P  L  A  P  S  S  K  S  T  S  G  G  T  A  A  L

      495      504      513      522      531      540
GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG AAC TCA
   ---
   G  C  L  V  K  D  Y  F  P  E  P  V  T  V  S  W  N  S

      549      558      567      576      585      594
GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA
   ---
   G  A  L  T  S  G  V  H  T  F  P  A  V  L  Q  S  S  G

```

**Fig. 13(D)**  
**(Sheet 1 of 3)**

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603	612	621	630	639	648
CTC TAC TCC CTC AGC	AGC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACC CAG				
---	---	---	---	---	---
L Y S L S S V V T V P S S S L G T Q					
657	666	675	684	693	702
ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA					
---	---	---	---	---	---
T Y I C N V N H K P S N T K V D K K					
711	720	729	738	747	756
GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT					
---	---	---	---	---	---
V E P K S C D K T H T C P P C P A P					
765	774	783	792	801	810
GAA GGC GGG CTG AAG ATC GCA GCC TTC AAC ATC CAG ACA TTT GGG GAG ACC AAG					
---	---	---	---	---	---
E G G L K I A A F N I Q T F G E T K					
819	828	837	846	855	864
ATG TCC AAT GCC ACC CTC GTC AGC TAC ATT GTG CAG ATC CTG AGC CGC TAC GAC					
---	---	---	---	---	---
M S N A T L V S Y I V Q I L S R Y D					
873	882	891	900	909	918
ATC GCC CTG GTC CAG GAG GTC AGA GAC AGC CAC CTG ACT GCC GTG GGG AAG CTG					
---	---	---	---	---	---
I A L V Q E V R D S H L T A V G K L					
927	936	945	954	963	972
CTG GAC AAC CTC AAT CAG GAC GCA CCA GAC ACC TAT CAC TAC GTG GTC AGT GAG					
---	---	---	---	---	---
L D N L N Q D A P D T Y H Y V V S E					
981	990	999	1008	1017	1026
CCA CTG GGA CGG AAC AGC TAT AAG GAG CGC TAC CTG TTC GTG TAC AGG CCT GAC					
---	---	---	---	---	---
P L G R N S Y K E R Y L F V Y R P D					
1035	1044	1053	1062	1071	1080
CAG GTG TCT GCG GTG GAC AGC TAC TAC TAC GAT GAT GGC TGC GAG CCC TGC GGG					
---	---	---	---	---	---
Q V S A V D S Y Y Y D D G C E P C G					
1089	1098	1107	1116	1125	1134
AAC GAC ACC TTC AAC CGA GAG CCA GCC ATT GTC AGG TTC TTC TCC CGG TTC ACA					
---	---	---	---	---	---
N D T F N R E P A I V R F F S R F T					
1143	1152	1161	1170	1179	1188
GAG GTC AGG GAG TTT GCC ATT GTT CCC CTG CAT GCG GCC CCG GGG GAC GCA GTA					
---	---	---	---	---	---
E V R E F A I V P L H A A P G D A V					
1197	1206	1215	1224	1233	1242

**Fig. 13(D)**  
**(Sheet 2 of 3)**

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```

GCC GAG ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT GTC CAA GAG AAA TGG GGC
-----
A   E   I   D   A   L   Y   D   V   Y   L   D   V   Q   E   K   W   G

      1251      1260      1269      1278      1287      1296
TTG GAG GAC GTC ATG TTG ATG GGC GAC TTC AAT GCG GGC TGC AGC TAT GTG AGA
-----
L   E   D   V   M   L   M   G   D   F   N   A   G   C   S   Y   V   R

      1305      1314      1323      1332      1341      1350
CCC TCC CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC CCC ACC TTC CAG TGG CTG
-----
P   S   Q   W   S   S   I   R   L   W   T   S   P   T   F   Q   W   L

      1359      1368      1377      1386      1395      1404
ATC CCC GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG CAC TGT GCC TAT GAC AGG
-----
I   P   D   S   A   D   T   T   A   T   P   T   H   C   A   Y   D   R

      1413      1422      1431      1440      1449      1458
ATC GTG GTT GCA GGG ATG CTG CTC CGA GGG GCC GTT GTT CCC GAC TCG GCT CTT
-----
I   V   V   A   G   M   L   L   R   G   A   V   V   P   D   S   A   L

      1467      1476      1485      1494      1503      1512
CCC TTT AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC CAA CTG GCC CAA GCC ATC
-----
P   F   N   F   Q   A   A   Y   G   L   S   D   Q   L   A   Q   A   I

      1521      1530      1539      1548
AGT GAC CAC TAT CCA GTG GAG GTG ATG CTG AAG TGA 3'
-----
S   D   H   Y   P   V   E   V   M   L   K   *

```

***Fig. 13(D)***  
***(Sheet 3 of 3)***

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pAS102

LOCUS PAS102.DNA 1566 bp mRNA PRI 06-MAR-1995  
 DEFINITION Humanised HMFG1 Fab'2 fused to human DNase I (pAS102)  
 ACCESSION  
 NID  
 KEYWORDS DNase I.  
 SOURCE DNase I sequence is from assembled oligos (thus modified c/f  
 MHDNASE1.dna) (See Figure 2)  
 ORGANISM Homo sapiens  
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;  
 Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.  
 AUTHORS Shak,S., Capon,D.J., Hellmiss,R., Marsters,S.A. and Baker,C.L.  
 TITLE Recombinant human DNase I reduces the viscosity of cystic  
 fibrosis  
 sputum  
 JOURNAL Proc. Natl. Acad. Sci. U.S.A. 87 (23), 9188-9192 (1990)  
 MEDLINE 91067672  
 BASE COUNT 345 a 469 c 440 g 312 t  
 ORIGIN

```

1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGCCTCAGT GAAGGTGTCC
121 TGCAAGGCTT CTGGCTACAC CTTCAAGTGC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
481 GCCCTGGGCT GCCTGGTCAA GGAATACTTC CCCGAACCGG TGACGGTGTC GTGGAAGTCA
541 GCGCGCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
721 GACAAAACCT ACACATGCTG TGTGGAGTGC CCACCGTGCC CAGCACCTGA AGGGAGCGGC
781 GGGCTGAAGA TCGCAGCCTT CAACATCCAG ACATTTGGGG AGACCAAGAT GTCCAATGCC
841 ACCCTCGTCA GCTACATTGT GCAGATCCTG AGCCGCTACG ACATCGCCCT GGTCCAGGAG
901 GTCAGAGACA GCCACCTGAC TGCCGTGGGG AAGCTGCTGG ACAACCTCAA TCAGGACGCA
961 CCAGACACCT ATCACTACGT GGTCAAGTGA CCACTGGGAC GGAACAGCTA TAAGGAGCGC
1021 TACCTGTTCT TGTACAGGCC TGACCAGGTG TCTGCGGTGG ACAGCTACTA CTACGATGAT
1081 GGCTGCGAGC CCTGCGGGAA CGACACCTTC AACCAGAGAG CAGCCATTGT CAGGTTCTTC
1141 TCCCGGTTCA CAGAGGTCAG GGAGTTTGCC ATTGTTCCCC TGCATGCGGC CCCGGGGGAC
1201 GCAGTAGCCG AGATCGACGC TCTCTATGAC GTCTACCTGG ATGTCCAAGA GAAATGGGGC
1261 TTGGAGGACG TCATGTTGAT GGGCGACTTC AATGCGGGCT GCAGCTATGT GAGACCTCC
1321 CAGTGGTCAT CCATCCGCCT GTGGACAAGC CCCACCTTCC AGTGGCTGAT CCCCAGACAGC
1381 GCTGACACCA CAGCTACACC CACGCACTGT GCCTATGACA GGATCGTGGT TGCAGGGATG
1441 CTGCTCCGAG GGGCCGTTGT TCCCGACTCG GCTCTTCCCT TTAAGTTCCA GGCTGCCTAT
1501 GGCCTGAGTG ACCAACTGGC CCAAGCCATC AGTGACCACT ATCCAGTGGA GGTGATGCTG
1561 AAGTGA

```

//

*Fig. 14(A)*

# 59/113

LOCUS FDDNASE102 1566 BP SS-DNA SYN 23-MAR-2001  
 DEFINITION -  
 ACCESSION -  
 KEYWORDS -  
 SOURCE -  
 BASE COUNT 345 A 468 C 440 G 313 T 0 OTHER  
 ORIGIN -

```

    1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
   61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
  121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
  181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
  241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
  301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
  361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
  421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
  481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACCTA
  541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
  601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
  661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
  721 GACAAAACCTC ACACATGCTG TGTCGAGTGT CCACCGTGTC CAGCACCAGA GGGGAGCGGC
  781 GGGCTGAAGA TCGCAGCCTT CAACATCCAG ACATTTGGGG AGACCAAGAT GTCCAATGCC
  841 ACCCTCGTCA GCTACATTGT GCAGATCCTG AGCCGCTACG ACATCGCCCT GGTCCAGGAG
  901 GTCAGAGACA GCCACCTGAC TGCCGTGGGG AAGCTGCTGG ACAACCTCAA TCAGGACGCA
  961 CCAGACACCT ATCACTACGT GGTCACTGAG CCACTGGGAC GGAACAGCTA TAAGGAGCGC
 1021 TACCTGTTCG TGTACAGGCC TGACCAGGTG TCTGCGGTGG ACAGCTACTA CTACGATGAT
 1081 GGCTGCGAGC CCTGCGGGAA CGACACCTTC AACCGAGAGC CAGCCATTGT CAGGTTCTTC
 1141 TCCCGGTTCA CAGAGGTCAG GGAGTTTGCC ATTGTTCCCC TGCATGCGGC CCCGGGGGAC
 1201 GCAGTAGCCG AGATCGACGC TCTCTATGAC GTCTACCTGG ATGTCCAAGA GAAATGGGGC
 1261 TTGGAGGACG TCATGTTGAT GGGCGACTTC AATGCGGGCT GCAGCTATGT GAGACCTTCC
 1321 CAGTGGTCAT CCATCCGCCT GTGGACAAGC CCCACCTTCC AGTGGCTGAT CCCCAGACAGC
 1381 GCTGACACCA CAGCTACACC CACGCACTGT GCCTATGACA GGATCGTGGT TGCAGGGATG
 1441 CTGCTCCGAG GGGCCGTTGT TCCCGACTCG GCTCTTCCCT TTAACCTCCA GGCTGCCTAT
 1501 GGCCTGAGTG ACCAACTGGC CCAAGCCATC AGTGACCACT ATCCAGTGGA GGTGATGCTG
 1561 AAGTGA
  
```

//

***Fig. 14(B)***

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**pAS302**

LOCUS FDDNASE302 1575 BP SS-DNA SYN 29-AUG-2000  
 DEFINITION -  
 ACCESSION -  
 KEYWORDS -  
 SOURCE -  
 FEATURES Location/Qualifiers  
     frag 10..1575  
         /note="1 to 1566 of FdDNase102correct"  
 BASE COUNT 346 A 474 C 442 G 313 T 0 OTHER  
 ORIGIN -  
     1 GCCGCCACCA TGGGATGGAG CTGTATCATC CTCTTCTTGG TAGCAACAGC TACAGGTGTC  
     61 CACTCCCAGG TGCAGCTGGT GCAGTCTGGG GCAGAGGTGA AAAAGCCTGG GGCCTCAGTG  
    121 AAGGTGTCCT GCAAGGCTTC TGGCTACACC TTCAGTGCCT ACTGGATAGA GTGGGTGCGC  
    181 CAGGCTCCAG GAAAGGGCCT CGAGTGGGTC GGAGAGATTT TACCTGGAAG TAATAATTCT  
    241 AGATACAATG AGAAGTTCAA GGGCCGAGTG ACAGTCACTA GAGACACATC CACAAACACA  
    301 GCCTACATGG AGCTCAGCAG CCTGAGGTCT GAGGACACAG CCGTCTATTA CTGTGCAAGA  
    361 TCCTACGACT TTGCCTGGTT TGCTTACTGG GGCCAAGGGA CTCTGGTCAC AGTCTCCTCA  
    421 GCCTCCACCA AGGGCCCATC GGTCTTCCCC CTGGCACCCCT CCTCCAAGAG CACCTCTGGG  
    481 GGCACAGCGG CCCTGGGCTG CCTGGTCAAG GACTACTTCC CCGAACCAGT GACGGTGTCG  
    541 TGGAACTCAG GCGCCCTGAC CAGCGGCGTG CACACCTTCC CGGCTGTCCT ACAGTCCTCA  
    601 GGA CTCTACT CCCTCAGCAG CGTGGTGACC GTGCCCTCCA GCAGCTTGGG CACCCAGACC  
    661 TACATCTGCA ACGTGAATCA CAAGCCCAGC AACACCAAGG TGGACAAGAA AGTTGAGCCC  
    721 AAATCTTGTG ACAA AACTCA CACATGCTGT GTCGAGTGTC CACCGTGTCC AGCACCAGAG  
    781 GGGAGCGGCG GGCTGAAGAT CGCAGCCTTC AACATCCAGA CATTTGGGGA GACCAAGATG  
    841 TCCAATGCCA CCCTCGTCAG CTACATTGTG CAGATCCTGA GCCGCTACGA CATCGCCCTG  
    901 GTCCAGGAGG TCAGAGACAG CCACCTGACT GCCGTGGGGA AGCTGCTGGA CAACCTCAAT  
    961 CAGGACGCAC CAGACACCTA TCACTACGTG GTCAGTGAGC CACTGGGACG GAACAGCTAT  
 1021 AAGGAGCGCT ACCTGTTTCGT GTACAGGCCT GACCAGGTGT CTGCGGTGGA CAGCTACTAC  
 1081 TACGATGATG GCTGCGAGCC CTGCGGGAAC GACACCTTCA ACCGAGAGCC AGCCATTGTC  
 1141 AGGTTCTTCT CCCGGTTCAC AGAGGTCAGG GAGTTTGCCA TTGTTCCCTT GCATGCGGCC  
 1201 CCGGGGGACG CAGTAGCCGA GATCGACGCT CTCTATGACG TCTACCTGGA TGTCCAAGAG  
 1261 AAATGGGGCT TGGAGGACGT CATGTTGATG GGCGACTTCA ATGCGGGCTG CAGCTATGTG  
 1321 AGACCCTCCC AGTGGTCATC CATCCGCCGTG TGGACAAGCC CCACCTTCCA GTGGCTGATC  
 1381 CCCGACAGCG CTGACACCAC AGCTACACCC ACGCACTGTG CCTATGACAG GATCGTGTTT  
 1441 GCAGGGATGC TGCTCCGAGG GGCCGTTGTT CCCGACTCGG CTCTTCCCTT TAACTTCCAG  
 1501 GCTGCCTATG GCCTGAGTGA CCAACTGGCC CAAGCCATCA GTGACCACTA TCCAGTGGAG  
 1561 GTGATGCTGA AGTGA

//

***Fig. 14(C)***

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5'		9		18		27		36		45		54						
	ATG	GGA	TGG	AGC	TGT	ATC	ATC	CTC	TTC	TTG	GTA	GCA	ACA	GCT	ACA	GGT	GTC	CAC
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	M	G	W	S	C	I	I	L	F	L	V	A	T	A	T	G	V	H
		63		72		81		90		99		108						
	TCC	CAG	GTG	CAG	CTG	GTG	CAG	TCT	GGG	GCA	GAG	GTG	AAA	AAG	CCT	GGG	GCC	TCA
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	S	Q	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S
		117		126		135		144		153		162						
	GTG	AAG	GTG	TCC	TGC	AAG	GCT	TCT	GGC	TAC	ACC	TTC	AGT	GCC	TAC	TGG	ATA	GAG
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	V	K	V	S	C	K	A	S	G	Y	T	F	S	A	Y	W	I	E
		171		180		189		198		207		216						
	TGG	GTG	CGC	CAG	GCT	CCA	GGA	AAG	GGC	CTC	GAG	TGG	GTC	GGA	GAG	ATT	TTA	CCT
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	W	V	R	Q	A	P	G	K	G	L	E	W	V	G	E	I	L	P
		225		234		243		252		261		270						
	GGA	AGT	AAT	AAT	TCT	AGA	TAC	AAT	GAG	AAG	TTC	AAG	GGC	CGA	GTG	ACA	GTC	ACT
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	G	S	N	N	S	R	Y	N	E	K	F	K	G	R	V	T	V	T
		279		288		297		306		315		324						
	AGA	GAC	ACA	TCC	ACA	AAC	ACA	GCC	TAC	ATG	GAG	CTC	AGC	AGC	CTG	AGG	TCT	GAG
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	R	D	T	S	T	N	T	A	Y	M	E	L	S	S	L	R	S	E
		333		342		351		360		369		378						
	GAC	ACA	GCC	GTC	TAT	TAC	TGT	GCA	AGA	TCC	TAC	GAC	TTT	GCC	TGG	TTT	GCT	TAC
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	D	T	A	V	Y	Y	C	A	R	S	Y	D	F	A	W	F	A	Y
		387		396		405		414		423		432						
	TGG	GGC	CAA	GGG	ACT	CTG	GTC	ACA	GTC	TCC	TCA	GCC	TCC	ACC	AAG	GGC	CCA	TCG
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	W	G	Q	G	T	L	V	T	V	S	S	A	S	T	K	G	P	S
		441		450		459		468		477		486						
	GTC	TTC	CCC	CTG	GCA	CCC	TCC	TCC	AAG	AGC	ACC	TCT	GGG	GGC	ACA	GCG	GCC	CTG
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	V	F	P	L	A	P	S	S	K	S	T	S	G	G	T	A	A	L
		495		504		513		522		531		540						
	GGC	TGC	CTG	GTC	AAG	GAC	TAC	TTC	CCC	GAA	CCG	GTG	ACG	GTG	TCG	TGG	AAC	TCA
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	G	C	L	V	K	D	Y	F	P	E	P	V	T	V	S	W	N	S
		549		558		567		576		585		594						
	GGC	GCC	CTG	ACC	AGC	GGC	GTG	CAC	ACC	TTC	CCG	GCT	GTC	CTA	CAG	TCC	TCA	GGA
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	G	A	L	T	S	G	V	H	T	F	P	A	V	L	Q	S	S	G

**Fig. 14(D)**  
(Sheet 1 of 3)

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603	612	621	630	639	648
CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACC CAG					
L Y S L S S V V T V P S S S L G T Q					
657	666	675	684	693	702
ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA					
T Y I C N V N H K P S N T K V D K K					
711	720	729	738	747	756
GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC TGT GTG GAG TGC CCA CCG					
V E P K S C D K T H T C C V E C P P					
765	774	783	792	801	810
TGC CCA GCA CCT GAA GGG AGC GGC GGG CTG AAG ATC GCA GCC TTC AAC ATC CAG					
C P A P E G S G G L K I A A F N I Q					
819	828	837	846	855	864
ACA TTT GGG GAG ACC AAG ATG TCC AAT GCC ACC CTC GTC AGC TAC ATT GTG CAG					
T F G E T K M S N A T L V S Y I V Q					
873	882	891	900	909	918
ATC CTG AGC CGC TAC GAC ATC GCC CTG GTC CAG GAG GTC AGA GAC AGC CAC CTG					
I L S R Y D I A L V Q E V R D S H L					
927	936	945	954	963	972
ACT GCC GTG GGG AAG CTG CTG GAC AAC CTC AAT CAG GAC GCA CCA GAC ACC TAT					
T A V G K L L D N L N Q D A P D T Y					
981	990	999	1008	1017	1026
CAC TAC GTG GTC AGT GAG CCA CTG GGA CGG AAC AGC TAT AAG GAG CGC TAC CTG					
H Y V V S E P L G R N S Y K E R Y L					
1035	1044	1053	1062	1071	1080
TTC GTG TAC AGG CCT GAC CAG GTG TCT GCG GTG GAC AGC TAC TAC TAC GAT GAT					
F V Y R P D Q V S A V D S Y Y Y D D					
1089	1098	1107	1116	1125	1134
GGC TGC GAG CCC TGC GGG AAC GAC ACC TTC AAC CGA GAG CCA GCC ATT GTC AGG					
G C E P C G N D T F N R E P A I V R					
1143	1152	1161	1170	1179	1188
TTC TTC TCC CGG TTC ACA GAG GTC AGG GAG TTT GCC ATT GTT CCC CTG CAT GCG					
F F S R F T E V R E F A I V P L H A					
1197	1206	1215	1224	1233	1242

**Fig. 14(D)**  
**(Sheet 2 of 3)**

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GCC CCG GGG GAC GCA GTA GCC GAG ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT
---
A   P   G   D   A   V   A   E   I   D   A   L   Y   D   V   Y   L   D

      1251      1260      1269      1278      1287      1296
GTC CAA GAG AAA TGG GGC TTG GAG GAC GTC ATG TTG ATG GGC GAC TTC AAT GCG
---
V   Q   E   K   W   G   L   E   D   V   M   L   M   G   D   F   N   A

      1305      1314      1323      1332      1341      1350
GGC TGC AGC TAT GTG AGA CCC TCC CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC
---
G   C   S   Y   V   R   P   S   Q   W   S   S   I   R   L   W   T   S

      1359      1368      1377      1386      1395      1404
CCC ACC TTC CAG TGG CTG ATC CCC GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG
---
P   T   F   Q   W   L   I   P   D   S   A   D   T   T   A   T   P   T

      1413      1422      1431      1440      1449      1458
CAC TGT GCC TAT GAC AGG ATC GTG GTT GCA GGG ATG CTG CTC CGA GGG GCC GTT
---
H   C   A   Y   D   R   I   V   V   A   G   M   L   L   R   G   A   V

      1467      1476      1485      1494      1503      1512
GTT CCC GAC TCG GCT CTT CCC TTT AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC
---
V   P   D   S   A   L   P   F   N   F   Q   A   A   Y   G   L   S   D

      1521      1530      1539      1548      1557      1566
CAA CTG GCC CAA GCC ATC AGT GAC CAC TAT CCA GTG GAG GTG ATG CTG AAG TGA 3'
---
Q   L   A   Q   A   I   S   D   H   Y   P   V   E   V   M   L   K   *

```

***Fig. 14(D)***  
***(Sheet 3 of 3)***

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pAS103

LOCUS PAS103.DNA 1560 bp mRNA PRI 06-MAR-1995  
 DEFINITION Humanised HMFG1 Fab'2 fused to human DNase I (pAS103)  
 ACCESSION  
 NID  
 KEYWORDS DNase I.  
 SOURCE DNase I sequence is from assembled oligos (thus modified c/f MHDNASE1.dna)  
 ORGANISM Homo sapiens  
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata; Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.  
 AUTHORS Shak,S., Capon,D.J., Hellmiss,R., Marsters,S.A. and Baker,C.L.  
 TITLE Recombinant human DNase I reduces the viscosity of cystic fibrosis sputum  
 JOURNAL Proc. Natl. Acad. Sci. U.S.A. 87 (23), 9188-9192 (1990)  
 MEDLINE 91067672  
 BASE COUNT 344 a 468 c 436 g 312 t  
 ORIGIN

```

1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
121 TGCAAGGCTT CTGGCTACAC CTTCAAGTGC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
361 TTTGCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTG GTGGAAGTCA
541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCTCT AGGACTCTAC
601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
721 GACAAAATC ACACATGCTG TGTGGAGTGC CCACCGTGCC CAGCACCTGA AGGCGGGCTG
781 AAGATCGCAG CCTTCAACAT CCAGACATTT GGGGAGACCA AGATGTCCAA TGCCACCCTC
841 GTCAGCTACA TTGTGCAGAT CCTGAGCCGC TACGACATCG CCCTGGTCCA GGAGGTGAGA
901 GACAGCCACC TGACTGCCGT GGGGAAGCTG CTGGACAACC TCAATCAGGA CGCACCAGAC
961 ACCTATCACT ACGTGGTCAG TGAGCCACTG GGACGGAACA GCTATAAGGA GCGCTACCTG
1021 TTCGTGTACA GGCCTGACCA GGTGTCTGCG GTGGACAGCT ACTACTACGA TGATGGCTGC
1081 GAGCCCTGCG GGAACGACAC CTTCAACCGA GAGCCAGCCA TTGTCAAGTT CTTCTCCCGG
1141 TTCACAGAGG TCAGGGAGTT TGCCATTGTT CCCCTGCATG CGGCCCCGGG GGACGCAGTA
1201 GCCGAGATCG ACGCTCTCTA TGACGTCTAC CTGGATGTCC AAGAGAAATG GGGCTTGGAG
1261 GACGTCATGT TGATGGGCGA CTTCAATGCG GGCTGCAGCT ATGTGAGACC CTCCAGTGCG
1321 TCATCCATCC GCCTGTGGAC AAGCCCCACC TTCCAGTGGC TGATCCCCGA CAGCGCTGAC
1381 ACCACAGCTA CACCCACGCA CTGTGCCTAT GACAGGATCG TGGTTGCAGG GATGCTGCTC
1441 CGAGGGGCGG TTGTTCCCGA CTCGGCTCTT CCCTTAACT TCCAGGCTGC CTATGGCCTG
1501 AGTGACCAAC TGGCCCAAGC CATCAGTGAC CACTATCCAG TGGAGGTGAT GCTGAAGTGA

```

//

*Fig. 15(A)*

# 65/113

LOCUS FDDNASE103 1560 BP SS-DNA SYN 25-AUG-2000  
 DEFINITION -  
 ACCESSION -  
 KEYWORDS -  
 SOURCE -  
 FEATURES  
     frag Location/Qualifiers  
         join(1..>720,<793..1560)  
         /note="1 to 1560 of PAS103.dna [Split]"  
     frag 721..792  
         /note="1 to 72 of 103/107linker"  
     frag join(721..>771,<772..792)  
         /note="1 to 78 of 102linker [Split]"  
 BASE COUNT 344 A 467 C 436 G 313 T 0 OTHER  
 ORIGIN -  
     1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG  
     61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC  
     121 TGCAAGGCTT CTGGCTACAC CTTCACTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA  
     181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT  
     241 GAGAAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG  
     301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC  
     361 TTTGCCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC  
     421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG  
     481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTG GTGGAACCTCA  
     541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC  
     601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC  
     661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT  
     721 GACAAAATC ACACATGCTG TGTGAGTGT CCACCGTGTG CAGCACCAGA GGGCGGGCTG  
     781 AAGATCGCAG CTTTCAACAT CCAGACATTT GGGGAGACCA AGATGTCCAA TGCCACCCTC  
     841 GTCAGCTACA TTGTGCAGAT CCTGAGCCGC TACGACATCG CCCTGGTCCA GGAGGTCAGA  
     901 GACAGCCACC TGA CTGCGGT GGGGAAGCTG CTGGACAACC TCAATCAGGA CGCACCAGAC  
     961 ACCTATCACT ACGTGGTCAG TGAGCCACTG GGACGGAACA GCTATAAGGA GCGCTACCTG  
     1021 TTCGTGTACA GGCCTGACCA GGTGTCTGCG GTGGACAGCT ACTACTACGA TGATGGCTGC  
     1081 GAGCCCTGCG GGAACGACAC CTTCAACCGA GAGCCAGCCA TTGTGAGGTT CTTCTCCCGG  
     1141 TTCACAGAGG TCAGGGAGTT TGCCATTGTT CCCCTGCATG CGGCCCCGGG GGACGCAGTA  
     1201 GCCGAGATCG ACGCTCTCTA TGACGTCTAC CTGGATGTCC AAGAGAAATG GGGCTTGAGG  
     1261 GACGTCATGT TGATGGGCGA CTTCAATGCG GGCTGCAGCT ATGTGAGACC CTCCCAGTGG  
     1321 TCATCCATCC GCCTGTGGAC AAGCCCCACC TTCCAGTGGC TGATCCCCGA CAGCGCTGAC  
     1381 ACCACAGCTA CACCCACGCA CTGTGCCTAT GACAGGATCG TGGTTGCAGG GATGCTGCTC  
     1441 CGAGGGGCGG TTGTTCCCGA CTCGGCTCTT CCCTTTAACT TCCAGGCTGC CTATGGCCTG  
     1501 AGTGACCAAC TGGCCCAAGC CATCAGTGAC CACTATCCAG TGGAGGTGAT GCTGAAGTGA

//

**Fig. 15(B)**

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LOCUS FDDNASE103 1569 BP SS-DNA SYN 29-AUG-2000  
 DEFINITION -  
 ACCESSION -  
 KEYWORDS -  
 SOURCE -  
 FEATURES Location/Qualifiers  
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         /note="1 to 1560 of FdDNase103correct"  
     frag join(10..>729,<802..1569)  
         /note="1 to 1560 of PAS103.dna [Split]"  
     frag 730..801  
         /note="1 to 72 of 103/107linker"  
     frag join(730..>780,<781..801)  
         /note="1 to 78 of 102linker [Split]"  
 BASE COUNT 345 A 473 C 438 G 313 T 0 OTHER  
 ORIGIN -  
     1 GCCGCCACCA TGGGATGGAG CTGTATCATC CTCTTCTTGG TAGCAACAGC TACAGGTGTC  
     61 CACTCCAGG TGCAGCTGGT GCAGTCTGGG GCAGAGGTGA AAAAGCCTGG GGCCTCAGTG  
     121 AAGGTGTCCT GCAAGGCTTC TGGCTACACC TTCAGTGCCT ACTGGATAGA GTGGGTGCGC  
     181 CAGGCTCCAG GAAAGGGCCT CGAGTGGGTC GGAGAGATTT TACCTGGAAG TAATAATTCT  
     241 AGATACAATG AGAAGTTCAA GGGCCGAGTG ACAGTCACTA GAGACACATC CACAAACACA  
     301 GCCTACATGG AGCTCAGCAG CCTGAGGTCT GAGGACACAG CCGTCTATTA CTGTGCAAGA  
     361 TCCTACGACT TTGCCTGGTT TGCTTACTGG GGCCAAGGGA CTCTGGTCAC AGTCTCCTCA  
     421 GCCTCCACCA AGGGCCCATC GGTCTTCCCC CTGGCACCCCT CCTCCAAGAG CACCTCTGGG  
     481 GGCACAGCGG CCCTGGGCTG CCTGGTCAAG GACTACTTCC CCGAACCAGG GACGGTGTCG  
     541 TGGAAGTCAG GCGCCCTGAC CAGCGGCGTG CACACCTTCC CGGCTGTCCT ACAGTCCTCA  
     601 GGAAGTCTACT CCCTCAGCAG CGTGGTGACC GTGCCCTCCA GCAGCTTGGG CACCCAGACC  
     661 TACATCTGCA ACGTGAATCA CAAGCCCAGC AACACCAAGG TGGACAAGAA AGTTGAGCCC  
     721 AAATCTTGTTG AAAAACTCA CACATGCTGT GTCGAGTGTC CACCGTGTCC AGCACCAGAG  
     781 GGCAGGCTGA AGATCGCAGC CTTCAACATC CAGACATTTG GGGAGACCAA GATGTCCAAT  
     841 GCCACCCCTCG TCAGCTACAT TGTGCAGATC CTGAGCCGCT ACGACATCGC CCTGGTCCAG  
     901 GAGGTCAGAG ACAGCCACCT GACTGCCGTG GGGAGAGCTG TGGACAACCT CAATCAGGAC  
     961 GCACCAGACA CCTATCACTA CGTGGTCACT GAGCCACTGG GACGGAACAG CTATAAGGAG  
     1021 CGCTACCTGT TCGTGTACAG GCCTGACCAG GTGTCTGCGG TGGACAGCTA CTACTACGAT  
     1081 GATGGCTGCG AGCCCTGCGG GAACGACACC TTCAACCGAG AGCCAGCCAT TGTGAGGTTT  
     1141 TTCTCCCGGT TCACAGAGGT CAGGGAGTTT GCCATTGTTT CCCTGCATGC GGCCCCGGGG  
     1201 GACGCAGTAG CCGAGATCGA CGCTCTCTAT GACGTCTACC TGGATGTCCA AGAGAAATGG  
     1261 GGCTTGAGAG ACGTCATGTT GATGGGCGAC TTCAATGCGG GCTGCAGCTA TGTGAGACCC  
     1321 TCCCAGTGGT CATCCATCCG CCTGTGGACA AGCCCCACCT TCCAGTGGCT GATCCCCGAC  
     1381 AGCGCTGACA CCACAGCTAC ACCACGCGAC TGTGCCTATG ACAGGATCGT GGTTCAGGGG  
     1441 ATGCTGCTCC GAGGGGCCGT TGTTCACGAC TCGGCTCTTC CCTTTAACTT CCAGGCTGCC  
     1501 TATGGCCTGA GTGACCAACT GGCCCAAGCC ATCAGTGACC ACTATCCAGT GGAGGTGATG  
     1561 CTGAAGTGA

//

*Fig. 15(C)*

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```

      9      18      27      36      45      54
5'  ATG GGA TGG AGC TGT ATC ATC CTC TTC TTG GTA GCA ACA GCT ACA GGT GTC CAC
    ---
    M  G  W  S  C  I  I  L  F  L  V  A  T  A  T  G  V  H

      63      72      81      90      99      108
TCC CAG GTG CAG CTG GTG CAG TCT GGG GCA GAG GTG AAA AAG CCT GGG GCC TCA
    ---
    S  Q  V  Q  L  V  Q  S  G  A  E  V  K  K  P  G  A  S

      117      126      135      144      153      162
GTG AAG GTG TCC TGC AAG GCT TCT GGC TAC ACC TTC AGT GCC TAC TGG ATA GAG
    ---
    V  K  V  S  C  K  A  S  G  Y  T  F  S  A  Y  W  I  E

      171      180      189      198      207      216
TGG GTG CGC CAG GCT CCA GGA AAG GGC CTC GAG TGG GTC GGA GAG ATT TTA CCT
    ---
    W  V  R  Q  A  P  G  K  G  L  E  W  V  G  E  I  L  P

      225      234      243      252      261      270
GGA AGT AAT AAT TCT AGA TAC AAT GAG AAG TTC AAG GGC CGA GTG ACA GTC ACT
    ---
    G  S  N  N  S  R  Y  N  E  K  F  K  G  R  V  T  V  T

      279      288      297      306      315      324
AGA GAC ACA TCC ACA AAC ACA GCC TAC ATG GAG CTC AGC AGC CTG AGG TCT GAG
    ---
    R  D  T  S  T  N  T  A  Y  M  E  L  S  S  L  R  S  E

      333      342      351      360      369      378
GAC ACA GCC GTC TAT TAC TGT GCA AGA TCC TAC GAC TTT GCC TGG TTT GCT TAC
    ---
    D  T  A  V  Y  Y  C  A  R  S  Y  D  F  A  W  F  A  Y

      387      396      405      414      423      432
TGG GGC CAA GGG ACT CTG GTC ACA GTC TCC TCA GCC TCC ACC AAG GGC CCA TCG
    ---
    W  G  Q  G  T  L  V  T  V  S  S  A  S  T  K  G  P  S

      441      450      459      468      477      486
GTC TTC CCC CTG GCA CCC TCC TCC AAG AGC ACC TCT GGG GGC ACA GCG GCC CTG
    ---
    V  F  P  L  A  P  S  S  K  S  T  S  G  G  T  A  A  L

      495      504      513      522      531      540
GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG AAC TCA
    ---
    G  C  L  V  K  D  Y  F  P  E  P  V  T  V  S  W  N  S

      549      558      567      576      585      594
GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA
    ---
    G  A  L  T  S  G  V  H  T  F  P  A  V  L  Q  S  S  G

```

**Fig. 15(D)**  
**(Sheet 1 of 3)**

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603	612	621	630	639	648
CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACC CAG					
---	---	---	---	---	---
L Y S L S S V V T V P S S S L G T Q					
657	666	675	684	693	702
ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA					
---	---	---	---	---	---
T Y I C N V N H K P S N T K V D K K					
711	720	729	738	747	756
GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC TGT GTG GAG TGC CCA CCG					
---	---	---	---	---	---
V E P K S C D K T H T C C V E C P P					
765	774	783	792	801	810
TGC CCA GCA CCT GAA GGC GGG CTG AAG ATC GCA GCC TTC AAC ATC CAG ACA TTT					
---	---	---	---	---	---
C P A P E G G L K I A A F N I Q T F					
819	828	837	846	855	864
GGG GAG ACC AAG ATG TCC AAT GCC ACC CTC GTC AGC TAC ATT GTG CAG ATC CTG					
---	---	---	---	---	---
G E T K M S N A T L V S Y I V Q I L					
873	882	891	900	909	918
AGC CGC TAC GAC ATC GCC CTG GTC CAG GAG GTC AGA GAC AGC CAC CTG ACT GCC					
---	---	---	---	---	---
S R Y D I A L V Q E V R D S H L T A					
927	936	945	954	963	972
GTG GGG AAG CTG CTG GAC AAC CTC AAT CAG GAC GCA CCA GAC ACC TAT CAC TAC					
---	---	---	---	---	---
V G K L L D N L N Q D A P D T Y H Y					
981	990	999	1008	1017	1026
GTG GTC AGT GAG CCA CTG GGA CGG AAC AGC TAT AAG GAG CGC TAC CTG TTC GTG					
---	---	---	---	---	---
V V S E P L G R N S Y K E R Y L F V					
1035	1044	1053	1062	1071	1080
TAC AGG CCT GAC GAG GTG TCT GCG GTG GAC AGC TAC TAC TAC GAT GAT GGC TGC					
---	---	---	---	---	---
Y R P D Q V S A V D S Y Y Y D D G C					
1089	1098	1107	1116	1125	1134
GAG CCC TGC GGG AAC GAC ACC TTC AAC CGA GAG CCA GCC ATT GTC AGG TTC TTC					
---	---	---	---	---	---
E P C G N D T F N R E P A I V R F F					
1143	1152	1161	1170	1179	1188
TCC CGG TTC ACA GAG GTC AGG GAG TTT GCC ATT GTT CCC CTG CAT GCG GCC CCG					
---	---	---	---	---	---
S R F T E V R E F A I V P L H A A P					
1197	1206	1215	1224	1233	1242

**Fig. 15(D)**  
**(Sheet 2 of 3)**

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```

GGG GAC GCA GTA GCC GAG ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT GTC CAA
---
G   D   A   V   A   E   I   D   A   L   Y   D   V   Y   L   D   V   Q

      1251      1260      1269      1278      1287      1296
GAG AAA TGG GGC TTG GAG GAC GTC ATG TTG ATG GGC GAC TTC AAT GCG GGC TGC
---
E   K   W   G   L   E   D   V   M   L   M   G   D   F   N   A   G   C

      1305      1314      1323      1332      1341      1350
AGC TAT GTG AGA CCC TCC CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC CCC ACC
---
S   Y   V   R   P   S   Q   W   S   S   I   R   L   W   T   S   P   T

      1359      1368      1377      1386      1395      1404
TTC CAG TGG CTG ATC CCC GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG CAC TGT
---
F   Q   W   L   I   P   D   S   A   D   T   T   A   T   P   T   H   C

      1413      1422      1431      1440      1449      1458
GCC TAT GAC AGG ATC GTG GTT GCA GGG ATG CTG CTC CGA GGG GCC GTT GTT CCC
---
A   Y   D   R   I   V   V   A   G   M   L   L   R   G   A   V   V   P

      1467      1476      1485      1494      1503      1512
GAC TCG GCT CTT CCC TTT AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC CAA CTG
---
D   S   A   L   P   F   N   F   Q   A   A   Y   G   L   S   D   Q   L

      1521      1530      1539      1548      1557
GCC CAA GCC ATC AGT GAC CAC TAT CCA GTG GAG GTG ATG CTG AAG TGA 3'
---
A   Q   A   I   S   D   H   Y   P   V   E   V   M   L   K   *

```

***Fig. 15(D)***  
***(Sheet 3 of 3)***

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pAS104

LOCUS PAS104.DNA 1560 bp mRNA PRI 06-MAR-1995  
 DEFINITION Humanised HMFG1 Fab'2 fused to human DNase I (pAS104)  
 Position 924 G to A by ggg to gag  
 Linker GR instead of GG (position 777)  
 ACCESSION  
 NID  
 KEYWORDS DNase I.  
 SOURCE DNase I sequence is from assembled oligos (thus modified c/f  
 MHDNASE1.dna)  
 ORGANISM Homo sapiens  
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;  
 Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.  
 AUTHORS Shak,S., Capon,D.J., Hellmiss,R., Marsters,S.A. and Baker,C.L.  
 TITLE Recombinant human DNase I reduces the viscosity of cystic  
 fibrosis  
 sputum  
 JOURNAL Proc. Natl. Acad. Sci. U.S.A. 87 (23), 9188-9192 (1990)  
 MEDLINE 91067672  
 BASE COUNT 346 a 468 c 434 g 312 t  
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1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCAG
61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
481 GCCCTGGGCT GCCTGGTCAA GGACTIONC CCCGAACCGG TGACGGTGTC GTGGAACCTCA
541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
601 TCCCTCAGCA CGGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
721 GACAAAACCTC ACACATGCTG TGTGGAGTGC CCACCGTGCC CAGCACCTGA AGGCAGGCTG
781 AAGATCGCAG CCTTCAACAT CCAGACATTT GGGGAGACCA AGATGTCCAA TGCCACCCTC
841 GTCAGCTACA TTGTGCAGAT CCTGAGCCGC TACGACATCG CCCTGGTCCA GGAGGTCAGA
901 GACAGCCACC TGACTIONC GGTGAAGCTG CTGGACAACC TCAATCAGGA CGCACCAGAC
961 ACCTATCACT ACGTGGTCAG TGAGCCACTG GGACGGAACA GCTATAAGGA GCGCTACCTG
1021 TTCGTGTACA GGCCTGACCA GGTGTCGCG GTGGACAGCT ACTACTACGA TGATGGCTGC
1081 GAGCCCTGCG GGAACGACAC CTTCAACCGA GAGCCAGCCA TTGTCAGGTT CTTCTCCCGG
1141 TTCACAGAGG TCAGGGAGTT TGCCATTGTT CCCCTGCATG CGGCCCCGGG GGACGCAGTA
1201 GCCGAGATCG ACGCTCTCTA TGACGTCTAC CTGGATGTCC AAGAGAAATG GGGCTTGGAG
1261 GACGTCATGT TGATGGGCGA CTTCAATGCG GGCTGCAGCT ATGTGAGACC CTCCCAGTGG
1321 TCATCCATCC GCCTGTGGAC AAGCCCCACC TTCCAGTGGC TGATCCCCGA CAGCGCTGAC
1381 ACCACAGCTA CACCCACGCA CTGTGCCTAT GACAGGATCG TGGTTGCAGG GATGCTGCTC
1441 CGAGGGGCCG TTGTTCCCGA CTCGGCTCTT CCCTTTAACT TCCAGGCTGC CTATGGCCTG
1501 AGTGACCAAC TGGCCCAAGC CATCAGTGAC CACTATCCAG TGGAGGTGAT GCTGAAGTGA

```

//

*Fig. 16(A)*

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LOCUS FDDNASE104 1560 BP SS-DNA SYN 25-AUG-2000  
 DEFINITION -  
 ACCESSION -  
 KEYWORDS -  
 SOURCE -  
 FEATURES Location/Qualifiers  
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           /note="1 to 1560 of PAS104.dna [Split]"  
     frag 721..792  
           /note="1 to 72 of 104linker"  
     frag join(721..>774,<776..792)  
           /note="1 to 72 of 103linker [Split]"  
     frag join(721..>771,<772..>774,<776..792)  
           /note="1 to 78 of 102linker [Split]"  
 BASE COUNT 346 A 467 C 434 G 313 T 0 OTHER  
 ORIGIN -  
     1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG  
     61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC  
     121 TGCAAGGCTT CTGGCTACAC CTTCAAGTGC TACTGGATAG AGTGGGTGCG CCAGGCTCCA  
     181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT  
     241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG  
     301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC  
     361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC  
     421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG  
     481 GCCCTGGGCT GCCTGGTCAA GGACTIONTC CCCGAACCGG TGACGGGTGTC GTGGAACCTA  
     541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC  
     601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC  
     661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT  
     721 GACAAAACCTC ACACATGCTG TGTCGAGTGT CCACCGTGTC CAGCACCAGA GGGCAGGCTG  
     781 AAGATCGCAG CTTTCAACAT CCAGACATTT GGGGAGACCA AGATGTCCAA TGCCACCCTC  
     841 GTCAGCTACA TTGTGCAGAT CCTGAGCCGC TACGACATCG CCCTGGTCCA GGAGGTCAGA  
     901 GACAGCCACC TGACTIONCGT GGAGAAGCTG CTGGACAACC TCAATCAGGA CGCACCAGAC  
     961 ACCTATCACT ACGTGGTCAG TGAGCCACTG GGACGGAACA GCTATAAGGA GCGCTACCTG  
     1021 TTCGTGTACA GGCCTGACCA GGTGTCTGCG GTGGACAGCT ACTACTACGA TGATGGCTGC  
     1081 GAGCCCTGCG GGAACGACAC CTTCAACCGA GAGCCAGCCA TTGTGAGGTT CTTCTCCCGG  
     1141 TTCACAGAGG TCAGGGAGTT TGCCATTGTT CCCCTGCATG CGGCCCCGGG GGACGCAGTA  
     1201 GCCGAGATCG ACGCTCTCTA TGACGTCTAC CTGGATGTCC AAGAGAAATG GGGCTTGGAG  
     1261 GACGTATGTT TGATGGGCGA CTTCAATGCG GGCTGCAGCT ATGTGAGACC CTCCAGTGG  
     1321 TCATCCATCC GCCTGTGGAC AAGCCCCACC TTCCAGTGGC TGATCCCCGA CAGCGCTGAC  
     1381 ACCACAGCTA CACCCACGCA CTGTGCCTAT GACAGGATCG TGGTTGCAGG GATGCTGCTC  
     1441 CGAGGGGCGG TTGTTCCCGA CTCGGCTCTT CCCTTTAACT TCCAGGCTGC CTATGGCCTG  
     1501 AGTGACCAAC TGGCCCAAGC CATCAGTGAC CACTATCCAG TGGAGGTGAT GCTGAAGTGA

//

**Fig. 16(B)**

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```

      9      18      27      36      45      54
5'  ATG GGA TGG AGC TGT ATC ATC CTC TTC TTG GTA GCA ACA GCT ACA GGT GTC CAC
    ---
    M  G  W  S  C  I  I  L  F  L  V  A  T  A  T  G  V  H

      63      72      81      90      99      108
TCC CAG GTG CAG CTG GTG CAG TCT GGG GCA GAG GTG AAA AAG CCT GGG GCC TCA
    ---
    S  Q  V  Q  L  V  Q  S  G  A  E  V  K  K  P  G  A  S

      117      126      135      144      153      162
GTG AAG GTG TCC TGC AAG GCT TCT GGC TAC ACC TTC AGT GCC TAC TGG ATA GAG
    ---
    V  K  V  S  C  K  A  S  G  Y  T  F  S  A  Y  W  I  E

      171      180      189      198      207      216
TGG GTG CGC CAG GCT CCA GGA AAG GGC CTC GAG TGG GTC GGA GAG ATT TTA CCT
    ---
    W  V  R  Q  A  P  G  K  G  L  E  W  V  G  E  I  L  P

      225      234      243      252      261      270
GGA AGT AAT AAT TCT AGA TAC AAT GAG AAG TTC AAG GGC CGA GTG ACA GTC ACT
    ---
    G  S  N  N  S  R  Y  N  E  K  F  K  G  R  V  T  V  T

      279      288      297      306      315      324
AGA GAC ACA TCC ACA AAC ACA GCC TAC ATG GAG CTC AGC AGC CTG AGG TCT GAG
    ---
    R  D  T  S  T  N  T  A  Y  M  E  L  S  S  L  R  S  E

      333      342      351      360      369      378
GAC ACA GCC GTC TAT TAC TGT GCA AGA TCC TAC GAC TTT GCC TGG TTT GCT TAC
    ---
    D  T  A  V  Y  Y  C  A  R  S  Y  D  F  A  W  F  A  Y

      387      396      405      414      423      432
TGG GGC CAA GGG ACT CTG GTC ACA GTC TCC TCA GCC TCC ACC AAG GGC CCA TCG
    ---
    W  G  Q  G  T  L  V  T  V  S  S  A  S  T  K  G  P  S

      441      450      459      468      477      486
GTC TTC CCC CTG GCA CCC TCC TCC AAG AGC ACC TCT GGG GGC ACA GCG GCC CTG
    ---
    V  F  P  L  A  P  S  S  K  S  T  S  G  G  T  A  A  L

      495      504      513      522      531      540
GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG AAC TCA
    ---
    G  C  L  V  K  D  Y  F  P  E  P  V  T  V  S  W  N  S

      549      558      567      576      585      594
GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA
    ---
    G  A  L  T  S  G  V  H  T  F  P  A  V  L  Q  S  S  G

```

**Fig. 16(C)**  
**(Sheet 1 of 3)**

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603	612	621	630	639	648
CTC TAC TCC	CTC AGC AGC	GTG GTG ACC	GTG CCC TCC	AGC AGC TTG	GGC ACC CAG
---	---	---	---	---	---
L Y S	L S S	V V T	V P S	S S S	L G T
---	---	---	---	---	---
657	666	675	684	693	702
ACC TAC ATC	TGC AAC GTG	AAT CAC AAG	CCC AGC AAC	ACC AAG GTG	GAC AAG AAA
---	---	---	---	---	---
T Y I	C N V	N H K	P S N	T K V	D K K
---	---	---	---	---	---
711	720	729	738	747	756
GTT GAG CCC	AAA TCT TGT	GAC AAA ACT	CAC ACA TGC	TGT GTG GAG	TGC CCA CCG
---	---	---	---	---	---
V E P	K S C	D K T	H T C	C V E	C P P
---	---	---	---	---	---
765	774	783	792	801	810
TGC CCA GCA	CCT GAA GGC	AGG CTG AAG	ATC GCA GCC	TTC AAC ATC	CAG ACA TTT
---	---	---	---	---	---
C P A	P E G	R L K	I A A	F N I	Q T F
---	---	---	---	---	---
819	828	837	846	855	864
GGG GAG ACC	AAG ATG TCC	AAT GCC ACC	CTC GTC AGC	TAC ATT GTG	CAG ATC CTG
---	---	---	---	---	---
G E T	K M S	N A T	L V S	Y I V	Q I L
---	---	---	---	---	---
873	882	891	900	909	918
AGC CGC TAC	GAC ATC GCC	CTG GTC CAG	GAG GTC AGA	GAC AGC CAC	CTG ACT GCC
---	---	---	---	---	---
S R Y	D I A	L V Q	E V R	D S H	L T A
---	---	---	---	---	---
927	936	945	954	963	972
GTG GAG AAG	CTG CTG GAC	AAC CTC AAT	CAG GAC GCA	CCA GAC ACC	TAT CAC TAC
---	---	---	---	---	---
V E K	L L D	N L N	Q D A	P D T	Y H Y
---	---	---	---	---	---
981	990	999	1008	1017	1026
GTG GTC AGT	GAG CCA CTG	GGA CGG AAC	AGC TAT AAG	GAG CGC TAC	CTG TTC GTG
---	---	---	---	---	---
V V S	E P L	G R N	S Y K	E R Y	L F V
---	---	---	---	---	---
1035	1044	1053	1062	1071	1080
TAC AGG CCT	GAC CAG GTG	TCT GCG GTG	GAC AGC TAC	TAC TAC GAT	GAT GGC TGC
---	---	---	---	---	---
Y R P	D Q V	S A V	D S Y	Y Y Y	D D G
---	---	---	---	---	---
1089	1098	1107	1116	1125	1134
GAG CCC TGC	GGG AAC GAC	ACC TTC AAC	CGA GAG CCA	GCC ATT GTC	AGG TTC TTC
---	---	---	---	---	---
E P C	G N D	T F N	R E P	A I V	R F F
---	---	---	---	---	---
1143	1152	1161	1170	1179	1188
TCC CGG TTC	ACA GAG GTC	AGG GAG TTT	GCC ATT GTT	CCC CTG CAT	GCG GCC CCG
---	---	---	---	---	---
S R F	T E V	R E F	A I V	P L H	A A P
---	---	---	---	---	---
1197	1206	1215	1224	1233	1242

**Fig. 16(C)**  
**(Sheet 2 of 3)**

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```

GGG GAC GCA GTA GCC GAG ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT GTC CAA
---
G   D   A   V   A   E   I   D   A   L   Y   D   V   Y   L   D   V   Q

      1251      1260      1269      1278      1287      1296
GAG AAA TGG GGC TTG GAG GAC GTC ATG TTG ATG GGC GAC TTC AAT GCG GGC TGC
---
E   K   W   G   L   E   D   V   M   L   M   G   D   F   N   A   G   C

      1305      1314      1323      1332      1341      1350
AGC TAT GTG AGA CCC TCC CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC CCC ACC
---
S   Y   V   R   P   S   Q   W   S   S   I   R   L   W   T   S   P   T

      1359      1368      1377      1386      1395      1404
TTC CAG TGG CTG ATC CCC GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG CAC TGT
---
F   Q   W   L   I   P   D   S   A   D   T   T   A   T   P   T   H   C

      1413      1422      1431      1440      1449      1458
GCC TAT GAC AGG ATC GTG GTT GCA GGG ATG CTG CTC CGA GGG GCC GTT GTT CCC
---
A   Y   D   R   I   V   V   A   G   M   L   L   R   G   A   V   V   P

      1467      1476      1485      1494      1503      1512
GAC TCG GCT CTT CCC TTT AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC CAA CTG
---
D   S   A   L   P   F   N   F   Q   A   A   Y   G   L   S   D   Q   L

      1521      1530      1539      1548      1557
GCC CAA GCC ATC AGT GAC CAC TAT CCA GTG GAG GTG ATG CTG AAG TGA 3'
---
A   Q   A   I   S   D   H   Y   P   V   E   V   M   L   K   *

```

***Fig. 16(C)***  
***(Sheet 3 of 3)***

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**pAS105**

LOCUS PAS105.DNA 1578 bp mRNA PRI 06-MAR-1995  
 DEFINITION Humanised HMFG1 Fab'2 fused to human DNase I with SV40  
 NLS(pAS105)  
 ACCESSION  
 NID  
 KEYWORDS DNase I.  
 SOURCE DNase I sequence is from assembled oligos (thus modified c/f  
 MHDNASE1.dna)  
 ORGANISM Homo sapiens  
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;  
 Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.  
 AUTHORS Shak, S., Capon, D.J., Hellmiss, R., Marsters, S.A. and Baker, C.L.  
 TITLE Recombinant human DNase I reduces the viscosity of cystic  
 fibrosis  
 sputum  
 JOURNAL Proc. Natl. Acad. Sci. U.S.A. 87 (23), 9188-9192 (1990)  
 MEDLINE 91067672  
 BASE COUNT 353 a 473 c 442 g 310 t  
 ORIGIN

```

1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
121 TGCAAGGCTT CTGGCTACAC CTTAGTGGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAAT
241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
481 GCCCTGGGCT GCCTGGTCAA GGACTIONTC CCCGAACCGG TGACGGTGTC GTGGAACCTCA
541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
721 GACAAAATC ACACATGCCC ACCGTGCCCCA GCACCTGAAG GCGGGCTGAA GATCGCAGCC
781 TTCAACATCC AGACATTTGG GGAGACCAAG ATGTCCAATG CCACCTCGT CAGCTACATT
841 GTGCAGATCC TGAGCCGCTA CGACATCGCC CTGGTCCAGG AGGTGAGAGA CAGCCACCTG
901 ACTGCCGTGG GGAAGCTGCT GGACAACCTC AATCAGGACG CACCAGACAC CTATCACTAC
961 GTGGTCAGTG AGCCACTGGG ACGGAACAGC TATAAGGAGC GCTACCTGTT CGTGTACAGG
1021 CCTGACCAGG TGTCTGCGGT GGACAGCTAC TACTACGATG ATGGCTGCGA GCCCTGCGGG
1081 AACGACACCT TCAACCGAGA GCCAGCCATT GTCAGGTTCT TCTCCCGGTT CACAGAGGTC
1141 AGGGAGTTTG CCATTGTTCC CCTGCATGCG GCCCCGGGGG ACGCAGTAGC CGAGATCGAC
1201 GCTCTATG ACGTCTACCT GGATGTCCAA GAGAAATGGG GCTTGGAGGA CGTCATGTTG
1261 ATGGGCGACT TCAATGCGGG CTGCAGCTAT GTGAGACCCT CCCAGTGCTC ATCCATCCGC
1321 CTGTGGACAA GCCCCACCTT CCAGTGGCTG ATCCCCGACA GCGCTGACAC CACAGCTACA
1381 CCCACGCACT GTGCCTATGA CAGGATCGTG GTTGAGGGA TGCTGCTCCG AGGGGCCGTT
1441 GTTCCCGACT CGGCTCTTCC CTTTAACTTC CAGGCTGCCT ATGGCCTGAG TGACCAACTG
1501 GCCAAGCCA TCAGTGACCA CTATCCAGTG GAGGTGATGC TGAAGGGGGG CGGACCCAAA
1561 AAGAAGCGCA AGGTTTGA

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//

└─→ NLS

**Fig. 17(A)**

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LOCUS FDDNASE105 1578 BP SS-DNA SYN 25-AUG-2000  
 DEFINITION -  
 ACCESSION -  
 KEYWORDS -  
 SOURCE -  
 FEATURES Location/Qualifiers  
     frag join(1..>720,<781..1578)  
           /note="1 to 1578 of PAS105.dna [Split]"  
     frag 721..780  
           /note="1 to 60 of 101/105linker"  
     frag join(721..>735,<736..>759,<760..>780)  
           /note="1 to 80 of 102linker [Split]"  
 BASE COUNT 353 A 471 C 443 G 311 T 0 OTHER  
 ORIGIN -  
     1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG  
    61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC  
 121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA  
 181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT  
 241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG  
 301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC  
 361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC  
 421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG  
 481 GCCCTGGGCT GCCTGGTCAA GGACTIONTC CCCGAACCGG TGACGGTGTC GTGGAACCTA  
 541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC  
 601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC  
 661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT  
 721 GACAAACTC ACACATGTCC ACCGTGTCCA GCACCAGAGG GCGGGCTGAA GATCGCAGCC  
 781 TTCAACATCC AGACATTTGG GGAGACCAAG ATGTCCAATG CCACCCTCGT CAGCTACATT  
 841 GTGCAGATCC TGAGCCGCTA CGACATCGCC CTGGTCCAGG AGGTCAGAGA CAGCCACCTG  
 901 ACTGCCGTGG GGAAGCTGCT GGACAACCTC AATCAGGACG CACCAGACAC CTATCACTAC  
 961 GTGGTCAGTG AGCCACTGGG ACGGAACAGC TATAAGGAGC GCTACCTGTT CGTGTACAGG  
 1021 CCTGACCAGG TGTCTGCGGT GGACAGCTAC TACTACGATG ATGGCTGCGA GCCCTGCGGG  
 1081 AACGACACCT TCAACCGAGA GCCAGCCATT GTCAGGTTCT TCTCCCGGTT CACAGAGGTC  
 1141 AGGGAGTTTG CCATTGTTCC CCTGCATGCG GCCCCGGGGG ACGCAGTAGC CGAGATCGAC  
 1201 GCTCTCTATG ACGTCTACCT GGATGTCCAA GAGAAATGGG GCTTGGAGGA CGTCATGTTG  
 1261 ATGGGCGACT TCAATGCGGG CTGCAGCTAT GTGAGACCCT CCCAGTGGTC ATCCATCCGC  
 1321 CTGTGGACAA GCCCCACCTT CCAGTGGCTG ATCCCCGACA GCGCTGACAC CACAGCTACA  
 1381 CCCACGCACT GTGCCTATGA CAGGATCGTG GTTGCAGGGA TGCTGCTCCG AGGGGCCGTT  
 1441 GTTCCCGACT CGGCTCTTCC CTTTAACTTC CAGGCTGCCT ATGGCCTGAG TGACCAACTG  
 1501 GCCCAAGCCA TCAGTGACCA CTATCCAGTG GAGGTGATGC TGAAGGGGGG CGGACCCAAA  
 1561 AAGAAGCGCA AGGTTTGA

//

*Fig. 17(B)*

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```

LOCUS       FDDNASE105      1587 BP SS-DNA                      SYN          29-AUG-2000
DEFINITION  -
ACCESSION   -
KEYWORDS     -
SOURCE      -
FEATURES             Location/Qualifiers
     frag              10..1587
                        /note="1 to 1578 of FdDNase105correct"
     frag              join(10..>729,<790..1587)
                        /note="1 to 1578 of PAS105.dna [Split]"
     frag              730..789
                        /note="1 to 60 of 101/105linker"
     frag              join(730..>744,<745..>768,<769..>789)
                        /note="1 to 80 of 102linker [Split]"
BASE COUNT      354 A      477 C      445 G      311 T          0 OTHER
ORIGIN        -
      1  GCCGCCACCA  TGGGATGGAG  CTGTATCATC  CTCTTCTTGG  TAGCAACAGC  TACAGGTGTC
     61  CACTCCCAGG  TGCAGCTGGT  GCAGTCTGGG  GCAGAGGTGA  AAAAGCCTGG  GGCCTCAGTG
    121  AAGGTGTCCT  GCAAGGCTTC  TGGCTACACC  TTCAGTGCCCT  ACTGGATAGA  GTGGGTGCGC
    181  CAGGCTCCAG  GAAAGGGCCT  CGAGTGGGTC  GGAGAGATTT  TACCTGGAAG  TAATAATTCT
    241  AGATACAATG  AGAAGTTCAA  GGGCCGAGTG  ACAGTCACTA  GAGACACATC  CACAAACACA
    301  GCCTACATGG  AGCTCAGCAG  CCTGAGGTCT  GAGGACACAG  CCGTCTATTA  CTGTGCAAGA
    361  TCCTACGACT  TTGCCTGGTT  TGCTTACTGG  GGCCAAGGGA  CTCTGGTCAC  AGTCTCCTCA
    421  GCCTCCACCA  AGGGCCCATC  GGTCTTCCCC  CTGGCACCCCT  CCTCCAAGAG  CACCTCTGGG
    481  GGCACAGCGG  CCCTGGGCTG  CCTGGTCAAG  GACTACTTCC  CCGAACCGGT  GACGGTGTCTG
    541  TGGAATCAG  GCGCCCTGAC  CAGCGGCGTG  CACACCTTCC  CGGCTGTCCCT  ACAGTCCTCA
    601  GGA CTCTACT  CCCTCAGCAG  CGTGGTGACC  GTGCCCTCCA  GCAGCTTGGG  CACCCAGACC
    661  TACATCTGCA  ACGTGAATCA  CAAGCCCAGC  AACACCAAGG  TGGACAAGAA  AGTTGAGCCC
    721  AAATCTTGTG  ACAAAACTCA  CACATGTCCA  CCGTGTCCAG  CACCAGAGGG  CGGGCTGAAG
    781  ATCGCAGCCT  TCAACATCCA  GACATTTGGG  GAGACCAAGA  TGTCCAATGC  CACCCTCGTC
    841  AGCTACATTG  TGCAGATCCT  GAGCCGCTAC  GACATCGCCC  TGGTCCAGGA  GGTCAGAGAC
    901  AGCCACCTGA  CTGCCGTGGG  GAAGCTGCTG  GACAACCTCA  ATCAGGACGC  ACCAGACACC
    961  TATCACTACG  TGGTCAGTGA  GCCACTGGGA  CGGAACAGCT  ATAAGGAGCG  CTACCTGTTC
   1021  GTGTACAGGC  CTGACCAGGT  GTCTGCGGTG  GACAGCTACT  ACTACGATGA  TGGCTGCGAG
   1081  CCCTGCGGGA  ACGACACCTT  CAACCGAGAG  CCAGCCATTG  TCAGGTTCTT  CTCCCGGTTT
   1141  ACAGAGGTCA  GGGAGTTTGC  CATTGTTCCC  CTGCATGCGG  CCCCAGGGGA  CGCAGTAGCC
   1201  GAGATCGACG  CTCTCTATGA  CGTCTACCTG  GATGTCCAAG  AGAAATGGGG  CTTGGAGGAC
   1261  GTCATGTTGA  TGGGCGACTT  CAATGCGGGC  TGCAGCTATG  TGAGACCCTC  CCAGTGGTCA
   1321  TCCATCCGCC  TGTGGACAAG  CCCCACCTTC  CAGTGGCTGA  TCCCCGACAG  CGCTGACACC
   1381  ACAGCTACAC  CCACGCACTG  TGCCTATGAC  AGGATCGTGG  TTGCAGGGAT  GCTGCTCCGA
   1441  GGGGCCGTTG  TTCCCGACTC  GGCTCTTCCC  TTAACTTCC  AGGCTGCCTA  TGGCCTGAGT
   1501  GACCAACTGG  CCCAAGCCAT  CAGTGACCAC  TATCCAGTGG  AGGTGATGCT  GAAGGGGGGC
   1561  GGACCCAAAA  AGAAGCGCAA  GGTTTGA

```

//

## Fig. 17(C)

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```

      9      18      27      36      45      54
5'  ATG GGA TGG AGC TGT ATC ATC CTC TTC TTG GTA GCA ACA GCT ACA GGT GTC CAC
    ---
    M  G  W  S  C  I  I  L  F  L  V  A  T  A  T  G  V  H

      63      72      81      90      99      108
TCC CAG GTG CAG CTG GTG CAG TCT GGG GCA GAG GTG AAA AAG CCT GGG GCC TCA
    ---
    S  Q  V  Q  L  V  Q  S  G  A  E  V  K  K  P  G  A  S

      117      126      135      144      153      162
GTG AAG GTG TCC TGC AAG GCT TCT GGC TAC ACC TTC AGT GCC TAC TGG ATA GAG
    ---
    V  K  V  S  C  K  A  S  G  Y  T  F  S  A  Y  W  I  E

      171      180      189      198      207      216
TGG GTG CGC CAG GCT CCA GGA AAG GGC CTC GAG TGG GTC GGA GAG ATT TTA CCT
    ---
    W  V  R  Q  A  P  G  K  G  L  E  W  V  G  E  I  L  P

      225      234      243      252      261      270
GGA AGT AAT AAT TCT AGA TAC AAT GAG AAG TTC AAG GGC CGA GTG ACA GTC ACT
    ---
    G  S  N  N  S  R  Y  N  E  K  F  K  G  R  V  T  V  T

      279      288      297      306      315      324
AGA GAC ACA TCC ACA AAC ACA GCC TAC ATG GAG CTC AGC AGC CTG AGG TCT GAG
    ---
    R  D  T  S  T  N  T  A  Y  M  E  L  S  S  L  R  S  E

      333      342      351      360      369      378
GAC ACA GCC GTC TAT TAC TGT GCA AGA TCC TAC GAC TTT GCC TGG TTT GCT TAC
    ---
    D  T  A  V  Y  Y  C  A  R  S  Y  D  F  A  W  F  A  Y

      387      396      405      414      423      432
TGG GGC CAA GGG ACT CTG GTC ACA GTC TCC TCA GCC TCC ACC AAG GGC CCA TCG
    ---
    W  G  Q  G  T  L  V  T  V  S  S  A  S  T  K  G  P  S

      441      450      459      468      477      486
GTC TTC CCC CTG GCA CCC TCC TCC AAG AGC ACC TCT GGG GGC ACA GCG GCC CTG
    ---
    V  F  P  L  A  P  S  S  K  S  T  S  G  G  T  A  A  L

      495      504      513      522      531      540
GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG AAC TCA
    ---
    G  C  L  V  K  D  Y  F  P  E  P  V  T  V  S  W  N  S

      549      558      567      576      585      594
GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA
    ---
    G  A  L  T  S  G  V  H  T  F  P  A  V  L  Q  S  S  G

```

**Fig. 17(D)**  
**(Sheet 1 of 3)**

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603	612	621	630	639	648
CTC TAC TCC CTC AGC	AGC GTG GTG ACC GTG CCC	TCC AGC AGC TTG GGC ACC CAG			
---	---	---	---	---	---
L Y S L S S V V T V P S S S L G T Q					
657	666	675	684	693	702
ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA					
---	---	---	---	---	---
T Y I C N V N H K P S N T K V D K K					
711	720	729	738	747	756
GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT					
---	---	---	---	---	---
V E P K S C D K T H T C P P C P A P					
765	774	783	792	801	810
GAA GGC GGG CTG AAG ATC GCA GCC TTC AAC ATC CAG ACA TTT GGG GAG ACC AAG					
---	---	---	---	---	---
E G G L K I A A F N I Q T F G E T K					
819	828	837	846	855	864
ATG TCC AAT GCC ACC CTC GTC AGC TAC ATT GTG CAG ATC CTG AGC CGC TAC GAC					
---	---	---	---	---	---
M S N A T L V S Y I V Q I L S R Y D					
873	882	891	900	909	918
ATC GCC CTG GTC CAG GAG GTC AGA GAC AGC CAC CTG ACT GCC GTG GGG AAG CTG					
---	---	---	---	---	---
I A L V Q E V R D S H L T A V G K L					
927	936	945	954	963	972
CTG GAC AAC CTC AAT CAG GAC GCA CCA GAC ACC TAT CAC TAC GTG GTC AGT GAG					
---	---	---	---	---	---
L D N L N Q D A P D T Y H Y V V S E					
981	990	999	1008	1017	1026
CCA CTG GGA CGG AAC AGC TAT AAG GAG CGC TAC CTG TTG GTG TAC AGG CCT GAC					
---	---	---	---	---	---
P L G R N S Y K E R Y L F V Y R P D					
1035	1044	1053	1062	1071	1080
CAG GTG TCT GCG GTG GAC AGC TAC TAC TAC GAT GAT GGC TGC GAG CCC TGC GGG					
---	---	---	---	---	---
Q V S A V D S Y Y Y D D G C E P C G					
1089	1098	1107	1116	1125	1134
AAC GAC ACC TTC AAC CGA GAG CCA GCC ATT GTC AGG TTC TTC TCC CGG TTC ACA					
---	---	---	---	---	---
N D T F N R E P A I V R F F S R F T					
1143	1152	1161	1170	1179	1188
GAG GTC AGG GAG TTT GCC ATT GTT CCC CTG CAT GCG GCC CCG GGG GAC GCA GTA					
---	---	---	---	---	---
E V R E F A I V P L H A A P G D A V					
1197	1206	1215	1224	1233	1242

**Fig. 17(D)**  
**(Sheet 2 of 3)**

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```

GCC GAG ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT GTC CAA GAG AAA TGG GGC
-----
A   E   I   D   A   L   Y   D   V   Y   L   D   V   Q   E   K   W   G

      1251      1260      1269      1278      1287      1296
TTG GAG GAC GTC ATG TTG ATG GGC GAC TTC AAT GCG GGC TGC AGC TAT GTG AGA
-----
L   E   D   V   M   L   M   G   D   F   N   A   G   C   S   Y   V   R

      1305      1314      1323      1332      1341      1350
CCC TCC CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC CCC ACC TTC CAG TGG CTG
-----
P   S   Q   W   S   S   I   R   L   W   T   S   P   T   F   Q   W   L

      1359      1368      1377      1386      1395      1404
ATC CCC GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG CAC TGT GCC TAT GAC AGG
-----
I   P   D   S   A   D   T   T   A   T   P   T   H   C   A   Y   D   R

      1413      1422      1431      1440      1449      1458
ATC GTG GTT GCA GGG ATG CTG CTC CGA GGG GCC GTT GTT CCC GAC TCG GCT CTT
-----
I   V   V   A   G   M   L   L   R   G   A   V   V   P   D   S   A   L

      1467      1476      1485      1494      1503      1512
CCC TTT AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC CAA CTG GCC CAA GCC ATC
-----
P   F   N   F   Q   A   A   Y   G   L   S   D   Q   L   A   Q   A   I

      1521      1530      1539      1548      1557      1566
AGT GAC CAC TAT CCA GTG GAG GTG ATG CTG AAG GGG GGC GGA CCC AAA AAG AAG
-----
S   D   H   Y   P   V   E   V   M   L   K   G   G   G   P   K   K   K

      1575
CGC AAG GTT TGA 3'
-----
R   K   V   *

```

***Fig. 17(D)***  
***(Sheet 3 of 3)***

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**pAS106**

LOCUS PAS106.DNA 1596 bp mRNA PRI 06-MAR-1995  
 DEFINITION Humanised HMFG1 Fab'2 fused to human DNase I with SV40  
 NLS(pAS106)  
 ACCESSION  
 NID  
 KEYWORDS DNase I.  
 SOURCE DNase I sequence is from assembled oligos (thus modified c/f  
 MHDNASE1.dna)  
 ORGANISM Homo sapiens  
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;  
 Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.  
 AUTHORS Shak, S., Capon, D.J., Hellmiss, R., Marsters, S.A. and Baker, C.L.  
 TITLE Recombinant human DNase I reduces the viscosity of cystic  
 fibrosis  
 sputum  
 JOURNAL Proc. Natl. Acad. Sci. U.S.A. 87 (23), 9188-9192 (1990)  
 MEDLINE 91067672  
 BASE COUNT 355 a 475 c 452 g 314 t  
 ORIGIN

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1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
121 TGCAAGGCTT CTGGCTACAC CTTAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA
541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCTC AGGACTCTAC
601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
721 GACAAACTC ACACATGCTG TGTGGAGTGC CCACCGTGCC CAGCACCTGA AGGGAGCGGC
781 GGGCTGAAGA TCGCAGCCTT CAACATCCAG ACATTTGGGG AGACCAAGAT GTCCAATGCC
841 ACCCTCGTCA GCTACATTGT GCAGATCCTG AGCCGCTACG ACATCGCCCT GTCCAGGAG
901 GTCAGAGACA GCCACCTGAC TGCCGTGGGG AAGCTGCTGG ACAACCTCAA TCAGGACGCA
961 CCAGACACCT ATCACTACGT GGTCAGTGAG CCACTGGGAC GGAACAGCTA TAAGGAGCGC
1021 TACCTGTTCTG TGTACAGGCC TGACCAGGTG TCTGCGGTGG ACAGCTACTA CTACGATGAT
1081 GGCTGCGAGC CCTGCGGGAA CGACACCTTC AACCAGAGAGC CAGCCATTGT CAGGTTCTTC
1141 TCCCGTTTCA CAGAGGTCAG GGAGTTTGCC ATTGTTCCCC TGCATGCGGC CCCGGGGGAC
1201 GCAGTAGCCG AGATCGACGC TCTCTATGAC GTCTACCTGG ATGTCCAAGA GAAATGGGGC
1261 TTGGAGGACG TCATGTTGAT GGGCGACTTC AATGCGGGCT GCAGCTATGT GAGACCCTCC
1321 CAGTGGTCAT CCATCCGCCT GTGGACAAGC CCCACCTTCC AGTGGCTGAT CCCCAGACAGC
1381 GCTGACACCA CAGCTACACC CACGCACTGT GCCTATGACA GGATCGTGGT TGCAGGGATG
1441 CTGCTCCGAG GGGCCGTTGT TCCCGACTCG GCTCTTCCCT TTAACTTCCA GGCTGCCTAT
1501 GGCCTGAGTG ACCAAGTGGC CCAAGCCATC AGTGACCACT ATCCAGTGGA GGTGATGCTG
1561 AAGGGGGGCG GACCCAAAAA GAAGCGCAAG GTTTGA

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→ NLS

***Fig. 18(A)***

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LOCUS       FDDNASE106      1596 BP SS-DNA                      SYN      25-AUG-2000
DEFINITION   -
ACCESSION    -
KEYWORDS     -
SOURCE       -
FEATURES             Location/Qualifiers
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                       /note="1 to 1596 of PAS106.dna [Split]"
     frag              721..798
                       /note="1 to 78 of 102/106linker"
BASE COUNT    355 A      474 C      452 G      315 T          0 OTHER
ORIGIN        -
      1  ATGGGATGGA  GCTGTATCAT  CCTCTTCTTG  GTAGCAACAG  CTACAGGTGT  CCACTCCCAG
     61  GTGCAGCTGG  TGCAGTCTGG  GGCAGAGGTG  AAAAAGCCTG  GGGCCTCAGT  GAAGGTGTCC
    121  TGCAAGGCTT  CTGGCTACAC  CTTCAGTGCC  TACTGGATAG  AGTGGGTGCG  CCAGGCTCCA
    181  GGAAAGGGCC  TCGAGTGGGT  CGGAGAGATT  TTACCTGGAA  GTAATAATTC  TAGATACAAT
    241  GAGAAGTTCA  AGGGCCGAGT  GACAGTCACT  AGAGACACAT  CCACAAACAC  AGCCTACATG
    301  GAGCTCAGCA  GCCTGAGGTC  TGAGGACACA  GCCGTCTATT  ACTGTGCAAG  ATCCTACGAC
    361  TTTGCCTGGT  TTGCTTACTG  GGGCCAAGGG  ACTCTGGTCA  CAGTCTCCTC  AGCCTCCACC
    421  AAGGGCCCAT  CGGTCTTCCC  CCTGGCACCC  TCCTCCAAGA  GCACCTCTGG  GGGCACAGCG
    481  GCCCTGGGCT  GCCTGGTCAA  GGACTACTTC  CCCGAACCGG  TGACGGTGTC  GTGGAAC TCA
    541  GCGGCCCTGA  CCAGCGGCGT  GCACACCTTC  CCGGCTGTCC  TACAGTCCTC  AGGACTCTAC
    601  TCCCTCAGCA  GCGTGGTGAC  CGTGCCCTCC  AGCAGCTTGG  GCACCCAGAC  CTACATCTGC
    661  AACGTGAATC  ACAAGCCCAG  CAACACCAAG  GTGGACAAGA  AAGTTGAGCC  CAAATCTTGT
    721  GACAAAATC  ACACATGCTG  TGTCGAGTGT  CCACCGTGTC  CAGCACCAGA  GGGGAGCGGC
    781  GGGCTGAAGA  TCGCAGCCTT  CAACATCCAG  ACATTTGGGG  AGACCAAGAT  GTCCAATGCC
    841  ACCCTCGTCA  GCTACATTGT  GCAGATCCTG  AGCCGCTACG  ACATCGCCCT  GGTCCAGGAG
    901  GTCAGAGACA  GCCACCTGAC  TGCCGTGGGG  AAGCTGCTGG  ACAACCTCAA  TCAGGACGCA
    961  CCAGACACCT  ATCACTACGT  GGTCACTGAG  CCACTGGGAC  GGAACAGCTA  TAAGGAGCGC
   1021  TACCTGTTCG  TGTACAGGCC  TGACCAGGTG  TCTGCGGTGG  ACAGCTACTA  CTACGATGAT
   1081  GGCTGCGAGC  CCTGCGGGAA  CGACACCTTC  AACCGAGAGC  CAGCCATTGT  CAGGTTCTTC
   1141  TCCCGGTTCA  CAGAGGTCAG  GGAGTTTGCC  ATTGTTCCCC  TGCATGCGGC  CCCGGGGGAC
   1201  GCAGTAGCCG  AGATCGACGC  TCTCTATGAC  GTCTACCTGG  ATGTCCAAGA  GAAATGGGGC
   1261  TTGGAGGACG  TCATGTTGAT  GGGCGACTTC  AATGCGGGCT  GCAGCTATGT  GAGACCTTCC
   1321  CAGTGGTCAT  CCATCCGCCT  GTGGACAAGC  CCCACCTTCC  AGTGGCTGAT  CCCCAGACAGC
   1381  GCTGACACCA  CAGCTACACC  CACGCACTGT  GCCTATGACA  GGATCGTGGT  TGCAGGGATG
   1441  CTGCTCCGAG  GGGCCGTTGT  TCCCGACTCG  GCTCTTCCCT  TTAAC TTCCA  GGCTGCCTAT
   1501  GGCCTGAGTG  ACCAACTGGC  CCAAGCCATC  AGTGACCACT  ATCCAGTGGA  GGTGATGCTG
   1561  AAGGGGGGCG  GACCCAAAAA  GAAGCGCAAG  GTTTGA

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//

*Fig. 18(B)*

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LOCUS FDDNASE106 1605 BP SS-DNA SYN 29-AUG-2000  
 DEFINITION -  
 ACCESSION -  
 KEYWORDS -  
 SOURCE -  
 FEATURES Location/Qualifiers  
     frag 10..1605  
         /note="1 to 1596 of FdDNase106correct"  
     frag join(10..>729,<808..1605)  
         /note="1 to 1596 of PAS106.dna [Split]"  
     frag 730..807  
         /note="1 to 78 of 102/106linker"  
 BASE COUNT 356 A 480 C 454 G 315 T 0 OTHER  
 ORIGIN -  
     1 GCGGCCACCA TGGGATGGAG CTGTATCATC CTCTTCTTGG TAGCAACAGC TACAGGTGTC  
     61 CACTCCCAGG TGCAGCTGGT GCAGTCTGGG GCAGAGGTGA AAAAGCCTGG GGCCFCAGTG  
     121 AAGGTGTCCT GCAAGGCTTC TGGCTACACC TTCAGTGCCT ACTGGATAGA GTGGGTGCGC  
     181 CAGGCTCCAG GAAAGGGCCT CGAGTGGGTC GGAGAGATTT TACCTGGAAG TAATAATTCT  
     241 AGATACAATG AGAAGTTCAA GGGCCGAGTG ACAGTCACTA GAGACACATC CACAAACACA  
     301 GCCTACATGG AGCTCAGCAG CCTGAGGTCT GAGGACACAG CCGTCTATTA CTGTGCAAGA  
     361 TCCTACGACT TTGCCTGGTT TGCTTACTGG GGCCAAGGGA CTCTGGTCAC AGTCTCCTCA  
     421 GCCTCCACCA AGGGCCCATC GGTCTTCCCC CTGGCACCTT CCTCCAAGAG CACCTCTGGG  
     481 GGCACAGCGG CCCTGGGCTG CCTGGTCAAG GACTACTTCC CCGAACCGGT GACGGTGTGCG  
     541 TGGAACCTAG GCGCCCTGAC CAGCGGCGTG CACACCTTCC CGGCTGTCTT ACAGTCCTCA  
     601 GGA CTCTACT CCCTCAGCAG CGTGGTGACC GTGCCCTCCA GCAGCTTGGG CACCCAGACC  
     661 TACATCTGCA ACGTGAATCA CAAGCCCAGG AACACCAAGG TGGACAAGAA AGTTGAGCCC  
     721 AAATCTTGTG ACAAACTCA CACATGCTGT GTCGAGTGTC CACCGTGTCC AGCACCAGAG  
     781 GGGAGCGGCG GGCTGAAGAT CGCAGCCTTC AACATCCAGA CATTGGGGA GACCAAGATG  
     841 TCCAATGCCA CCCTCGTCAG CTACATTGTG CAGATCCTGA GCGGCTACGA CATCGCCCTG  
     901 GTCCAGGAGG TCAGAGACAG CCACCTGACT GCCGTGGGGA AGCTGCTGGA CAACCTCAAT  
     961 CAGGACGCAC CAGACACCTA TCACTACGTG GTCAGTGAGC CACTGGGACG GAACAGCTAT  
     1021 AAGGAGCGCT ACCTGTTCGT GTACAGGCCT GACCAGGTGT CTGCGGTGGA CAGCTACTAC  
     1081 TACGATGATG GCTGCGAGCC CTGCGGGAAC GACACCTTCA ACCGAGAGCC AGCCATTGTC  
     1141 AGGTTCTTCT CCCGGTTCAC AGAGGTCAGG GAGTTTGCCA TTGTTCCCTT GCATGCGGCC  
     1201 CCGGGGGACG CAGTAGCCGA GATCGACGCT CTCTATGACG TCTACCTGGA TGTCCAAGAG  
     1261 AAATGGGGCT TGGAGGACGT CATGTTGATG GGC GACTTCA ATGCGGGCTG CAGCTATGTG  
     1321 AGACCTTCCC AGTGCTCATC CATCCGCTTG TGGACAAGCC CCACCTTCCA GTGGCTGATC  
     1381 CCCGACAGCG CTGACACCAC AGCTACACCC ACGCACTGTG CCTATGACAG GATCGTGTTT  
     1441 GCAGGGATGC TGCTCCGAGG GGCCGTTGTT CCCGACTCGG CTCTTCCCTT TAACTTCCAG  
     1501 GCTGCCTATG GCCTGAGTGA CCAACTGGCC CAAGCCATCA GTGACCACTA TCCAGTGGAG  
     1561 GTGATGCTGA AGGGGGGCGG ACCCAAAAAG AAGCGCAAGG TTTGA

//

*Fig. 18(C)*

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9	18	27	36	45	54
ATG GGA TGG AGC TGT ATC ATC CTC TTC TTG GTA GCA ACA GCT ACA GGT GTC CAC					
-----					
M G W S C I I L F L V A T A T G V H					
63	72	81	90	99	108
TCC CAG GTG CAG CTG GTG CAG TCT GGG GCA GAG GTG AAA AAG CCT GGG GCC TCA					
-----					
S Q V Q L V Q S G A E V K K P G A S					
117	126	135	144	153	162
GTG AAG GTG TCC TGC AAG GCT TCT GGC TAC ACC TTC AGT GCC TAC TGG ATA GAG					
-----					
V K V S C K A S G Y T F S A Y W I E					
171	180	189	198	207	216
TGG GTG CGC CAG GCT CCA GGA AAG GGC CTC GAG TGG GTC GGA GAG ATT TTA CCT					
-----					
W V R Q A P G K G L E W V G E I L P					
225	234	243	252	261	270
GGA AGT AAT AAT TCT AGA TAC AAT GAG AAG TTC AAG GGC CGA GTG ACA GTC ACT					
-----					
G S N N S R Y N E K F K G R V T V T					
279	288	297	306	315	324
AGA GAC ACA TCC ACA AAC ACA GCC TAC ATG GAG CTC AGC AGC CTG AGG TCT GAG					
-----					
R D T S T N T A Y M E L S S L R S E					
333	342	351	360	369	378
GAC ACA GCC GTC TAT TAC TGT GCA AGA TCC TAC GAC TTT GCC TGG TTT GCT TAC					
-----					
D T A V Y Y C A R S Y D F A W F A Y					
387	396	405	414	423	432
TGG GGC CAA GGG ACT CTG GTC ACA GTC TCC TCA GCC TCC ACC AAG GGC CCA TCG					
-----					
W G Q G T L V T V S S A S T K G P S					
441	450	459	468	477	486
GTC TTC CCC CTG GCA CCC TCC TCC AAG AGC ACC TCT GGG GGC ACA GCG GCC CTG					
-----					
V F P L A P S S K S T S G G T A A L					
495	504	513	522	531	540
GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG AAC TCA					
-----					
G C L V K D Y F P E P V T V S W N S					
549	558	567	576	585	594
GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA					
-----					
G A L T S G V H T F P A V L Q S S G					

**Fig. 18(C)**  
**(Sheet 1 of 3)**

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603	612	621	630	639	648
CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACC CAG					
L Y S L S S V V T V P S S S L G T Q					
657	666	675	684	693	702
ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA					
T Y I C N V N H K P S N T K V D K K					
711	720	729	738	747	756
GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC TGT GTG GAG TGC CCA CCG					
V E P K S C D K T H T C C V E C P P					
765	774	783	792	801	810
TGC CCA GCA CCT GAA GGG AGC GGC GGG CTG AAG ATC GCA GCC TTC AAC ATC CAG					
C P A P E G S G G L K I A A F N I Q					
819	828	837	846	855	864
ACA TTT GGG GAG ACC AAG ATG TCC AAT GCC ACC CTC GTC AGC TAC ATT GTG CAG					
T F G E T K M S N A T L V S Y I V Q					
873	882	891	900	909	918
ATC CTG AGC CGC TAC GAC ATC GCC CTG GTC CAG GAG GTC AGA GAC AGC CAC CTG					
I L S R Y D I A L V Q E V R D S H L					
927	936	945	954	963	972
ACT GCC GTG GGG AAG CTG CTG GAC AAC CTC AAT CAG GAC GCA CCA GAC ACC TAT					
T A V G K L L D N L N Q D A P D T Y					
981	990	999	1008	1017	1026
CAC TAC GTG GTC AGT GAG CCA CTG GGA CGG AAC AGC TAT AAG GAG CGC TAC CTG					
H Y V V S E P L G R N S Y K E R Y L					
1035	1044	1053	1062	1071	1080
TTC GTG TAC AGG CCT GAC CAG GTG TCT GCG GTG GAC AGC TAC TAC TAC GAT GAT					
F V Y R P D Q V S A V D S Y Y Y D D					
1089	1098	1107	1116	1125	1134
GGC TGC GAG CCC TGC GGG AAC GAC ACC TTC AAC CGA GAG CCA GCC ATT GTC AGG					
G C E P C G N D T F N R E P A I V R					
1143	1152	1161	1170	1179	1188
TTC TTC TCC CGG TTC ACA GAG GTC AGG GAG TTT GCC ATT GTT CCC CTG CAT GCG					
F F S R F T E V R E F A I V P L H A					
1197	1206	1215	1224	1233	1242

**Fig. 18(C)**  
(Sheet 2 of 3)

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GCC CCG GGG GAC GCA GTA GCC GAG ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT
---
A   P   G   D   A   V   A   E   I   D   A   L   Y   D   V   Y   L   D

      1251      1260      1269      1278      1287      1296
GTC CAA GAG AAA TGG GGC TTG GAG GAC GTC ATG TTG ATG GGC GAC TTC AAT GCG
---
V   Q   E   K   W   G   L   E   D   V   M   L   M   G   D   F   N   A

      1305      1314      1323      1332      1341      1350
GGC TGC AGC TAT GTG AGA CCC TCC CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC
---
G   C   S   Y   V   R   P   S   Q   W   S   S   I   R   L   W   T   S

      1359      1368      1377      1386      1395      1404
CCC ACC TTC CAG TGG CTG ATC CCC GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG
---
P   T   F   Q   W   L   I   P   D   S   A   D   T   T   A   T   P   T

      1413      1422      1431      1440      1449      1458
CAC TGT GCC TAT GAC AGG ATC GTG GTT GCA GGG ATG CTG CTC CGA GGG GCC GTT
---
H   C   A   Y   D   R   I   V   V   A   G   M   L   L   R   G   A   V

      1467      1476      1485      1494      1503      1512
GTT CCC GAC TCG GCT CTT CCC TTT AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC
---
V   P   D   S   A   L   P   F   N   F   Q   A   A   Y   G   L   S   D

      1521      1530      1539      1548      1557      1566
CAA CTG GCC CAA GCC ATC AGT GAC CAC TAT CCA GTG GAG GTG ATG CTG AAG GGG
---
Q   L   A   Q   A   I   S   D   H   Y   P   V   E   V   M   L   K   G

      1575      1584      1593
GGC GGA CCC AAA AAG AAG CGC AAG GTT TGA 3'
---
G   G   P   K   K   K   R   K   V   *

```

***Fig. 18(C)***  
***(Sheet 3 of 3)***

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**pAS107**

LOCUS PAS107.DNA 1590 bp mRNA PRI 06-MAR-1995  
 DEFINITION Humanised HMFG1 Fab'2 fused to human DNase I with SV40  
 NLS (pAS107)  
 ACCESSION  
 NID  
 KEYWORDS DNase I.  
 SOURCE DNase I sequence is from assembled oligos (thus modified c/f  
 MHDNASE1.dna)  
 ORGANISM Homo sapiens  
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;  
 Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.  
 AUTHORS Shak,S., Capon,D.J., Hellmiss,R., Marsters,S.A. and Baker,C.L.  
 TITLE Recombinant human DNase I reduces the viscosity of cystic  
 fibrosis  
 sputum  
 JOURNAL Proc. Natl. Acad. Sci. U.S.A. 87 (23), 9188-9192 (1990)  
 MEDLINE 91067672  
 BASE COUNT 354 a 474 c 448 g 314 t  
 ORIGIN

```

1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
121 TGCAAGGCTT CTGGCTACAC CTTCAAGTGC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
481 GCCCTGGGCT GCCTGGTCAA GGAATACTTC CCCGAACCGG TGACGGTGTC GTGGAAGTCA
541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCTCT AGGACTCTAC
601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
721 GACAAAATC ACACATGCTG TGTGGAGTGC CCACCGTGCC CAGCACCTGA AGGCGGGCTG
781 AAGATCGCAG CCTTCAACAT CCAGACATTG GGGGAGACCA AGATGTCCAA TGCCACCCTC
841 GTCAGCTACA TTGTGCAGAT CCTGAGCCGC TACGACATCG CCCTGGTCCA GGAGGTGAGA
901 GACAGCCACC TGAATGCCGT GGGGAAGCTG CTGGACAACC TCAATCAGGA CGCACCAGAC
961 ACCTATCACT ACGTGGTCAG TGAGCCACTG GGACGGAACA GCTATAAGGA GCGCTACCTG
1021 TTCGTGTACA GGCCTGACCA GGTGTCTGCG GTGGACAGCT ACTACTACGA TGATGGCTGC
1081 GAGCCCTGCG GGAACGACAC CTTCAACCGA GAGCCAGCCA TTGTGAGGTT CTTCTCCCGG
1141 TTCACAGAGG TCAGGGAGTT TGCCATTGTT CCCCTGCATG CGGCCCCGGG GGACGCAGTA
1201 GCCGAGATCG ACGCTCTCTA TGACGTCTAC CTGGATGTCC AAGAGAAATG GGGCTTGAGG
1261 GACGTCATGT TGATGGGCGA CTTCAATGCG GGCTGCAGCT ATGTGAGACC CTCCCAGTGG
1321 TCATCCATCC GCCTGTGGAC AAGCCCCACC TTCCAGTGGC TGATCCCCGA CAGCGCTGAC
1381 ACCACAGCTA CACCCACGCA CTGTGCCTAT GACAGGATCG TGGTTGAGG GATGCTGCTC
1441 CGAGGGGGCC TTGTTCCCGA CTCGGCTCTT CCCTTTAACT TCCAGGCTGC CTATGGCCTG
1501 AGTGACCAAC TGGCCCAAGC CATCAGTGAC CACTATCCAG TGGAGGTGAT GCTGAAGGGG
1561 GGCGGACCCA AAAAGAAGCG CAAGGTTTGA

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//


***Fig. 19(A)***

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LOCUS FDDNASE107 1590 BP SS-DNA SYN 25-AUG-2000  
 DEFINITION -  
 ACCESSION -  
 KEYWORDS -  
 SOURCE -  
 FEATURES Location/Qualifiers  
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           /note="1 to 1590 of PAS107.dna [Split]"  
     frag 721..792  
           /note="1 to 72 of 103/107linker"  
     frag join(721..>771,<772..792)  
           /note="1 to 78 of 102linker [Split]"  
 BASE COUNT 354 A 473 C 448 G 315 T 0 OTHER  
 ORIGIN -  
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 61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC  
 121 TGCAAGGCTT CTGGCTACAC CTTCAAGTGC TACTGGATAG AGTGGGTGCG CCAGGCTCCA  
 181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT  
 241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG  
 301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC  
 361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC  
 421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG  
 481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAAC TCA  
 541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC  
 601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC  
 661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT  
 721 GACAAAATC ACACATGCTG TGTCGAGTGT CCACCGTGTC CAGCACCAGA GGGCGGGCTG  
 781 AAGATCGCAG CTTTCAACAT CCAGACATTT GGGGAGACCA AGATGTCCAA TGCCACCCCTC  
 841 GTCAGCTACA TTGTGCAGAT CCTGAGCCGC TACGACATCG CCCTGGTCCA GGAGGTCAGA  
 901 GACAGCCACC TGA CTGCGGT GGGGAAGCTG CTGGACAACC TCAATCAGGA CGCACCAGAC  
 961 ACCTATCACT ACGTGGTCAG TGAGCCACTG GGACGGAACA GCTATAAGGA GCGCTACCTG  
 1021 TTCGTGTACA GGCCTGACCA GGTGTCTGCG GTGGACAGCT ACTACTACGA TGATGGCTGC  
 1081 GAGCCCTGCG GGAACGACAC CTTCAACCGA GAGCCAGCCA TTGTCAGGTT CTTCTCCCGG  
 1141 TTCACAGAGG TCAGGGAGTT TGCCATTGTT CCCCTGCATG CGGCCCCGGG GGACGCAGTA  
 1201 GCCGAGATCG ACGCTCTCTA TGACGTCTAC CTGGATGTCC AAGAGAAATG GGGCTTGAGG  
 1261 GACGTCATGT TGATGGGCGA CTTCAATGCG GGCTGCAGCT ATGTGAGACC CTCCCAGTGG  
 1321 TCATCCATCC GCCTGTGGAC AAGCCCCACC TTCCAGTGGC TGATCCCCGA CAGCGCTGAC  
 1381 ACCACAGCTA CACCCACGCA CTGTGCCTAT GACAGGATCG TGGTTGCAGG GATGCTGCTC  
 1441 CGAGGGGCGG TTGTTCCCGA CTCGGCTCTT CCCTTTAACT TCCAGGCTGC CTATGGCCTG  
 1501 AGTGACCAAC TGGCCCAAGC CATCAGTGAC CACTATCCAG TGGAGGTGAT GCTGAAGGGG  
 1561 GGCGGACCCA AAAAGAAGCG CAAGGTTTGA

//

*Fig. 19(B)*

89/113

LOCUS FDDNASE107 1599 BP SS-DNA SYN 29-AUG-2000  
 DEFINITION -  
 ACCESSION -  
 KEYWORDS -  
 SOURCE -  
 FEATURES Location/Qualifiers  
     frag 10..1599  
         /note="1 to 1590 of FdDNase107correct"  
     frag join(10..>729,<802..1599)  
         /note="1 to 1590 of PAS107.dna [Split]"  
     frag 730..801  
         /note="1 to 72 of 103/107linker"  
     frag join(730..>780,<781..801)  
         /note="1 to 78 of 102linker [Split]"  
 BASE COUNT 355 A 479 C 450 G 315 T 0 OTHER  
 ORIGIN -  
     1 GCGGCCACCA TGGGATGGAG CTGTATCATC CTCTTCTTGG TAGCAACAGC TACAGGTGTC  
     61 CACTCCCAGG TGCAGCTGGT GCAGTCTGGG GCAGAGGTGA AAAAGCCTGG GGCCTCAGTG  
     121 AAGGTGTCCT GCAAGGCTTC TGGCTACACC TTCAGTGCCT ACTGGATAGA GTGGGTGCGC  
     181 CAGGCTCCAG GAAAGGGCCT CGAGTGGGTC GGAGAGATTT TACCTGGAAG TAATAATTCT  
     241 AGATACAATG AGAAGTTCAA GGGCCGAGTG ACAGTCACTA GAGACACATC CACAAACACA  
     301 GCCTACATGG AGCTCAGCAG CCTGAGGTCT GAGGACACAG CCGTCTATTA CTGTGCAAGA  
     361 TCCTACGACT TTGCCTGGTT TGCTTACTGG GGCCAAGGGA CTCTGGTCAC AGTCTCCTCA  
     421 GCCTCCACCA AGGGCCCATC GGTCTTCCCC CTGGCACCCCT CCTCCAAGAG CACCTCTGGG  
     481 GGCACAGCGG CCCTGGGCTG CCTGGTCAAG GACTACTTCC CCGAACCGGT GACGGTGTCTG  
     541 TGGAACCTCAG GCGCCCTGAC CAGCGGCGTG CACACCTTCC CGGCTGTCCT ACAGTCCTCA  
     601 GGAATCTACT CCCTCAGCAG CGTGGTGACC GTGCCCTCCA GCAGCTTGGG CACCCAGACC  
     661 TACATCTGCA ACGTGAATCA CAAGCCAGC AACACCAAGG TGGACAAGAA AGTTGAGCCC  
     721 AAATCTTGTG ACAAACCTCA CACATGCTGT GTCGAGTGTC CACCGTGTCC AGCACCAGAG  
     781 GCGGGGCTGA AGATCGCAGC CTTCAACATC CAGACATTTG GGGAGACCAA GATGTCCAAT  
     841 GCCACCCTCG TCAGCTACAT TGTGCAGATC CTGAGCCGCT ACGACATCGC CCTGGTCCAG  
     901 GAGGTCAGAG ACAGCCACCT GACTGCCGTG GGGGAAGCTGC TGGACAACCT CAATCAGGAC  
     961 GCACCAGACA CCTATCACTA CGTGGTCACT GAGCCACTGG GACGGAACAG CTATAAGGAG  
     1021 CGCTACCTGT TCGTGTACAG GCCTGACCAG GTGTCTGCGG TGGACAGCTA CTACTACGAT  
     1081 GATGGCTGCG AGCCCTGCGG GAACGACACC TTCAACCGAG AGCCAGCCAT TGTCAGGTTT  
     1141 TTCTCCCGGT TCACAGAGGT CAGGGAGTTT GCCATTGTTC CCCTGCATGC GGCCCCGGGG  
     1201 GACGCAGTAG CCGAGATCGA CGCTCTCTAT GACGTCTACC TGGATGTCCA AGAGAAATGG  
     1261 GGCTTGAGAG ACGTCATGTT GATGGGCGAC TTCAATGCGG GCTGCAGCTA TGTGAGACCC  
     1321 TCCAGTGGT CATCCATCCG CCTGTGGACA AGCCCCACCT TCCAGTGGCT GATCCCCGAC  
     1381 AGCGCTGACA CCACAGCTAC ACCCACGCAC TGTGCCTATG ACAGGATCGT GGTTCAGGG  
     1441 ATGCTGCTCC GAGGGGCCGT TGTTCCTGAC TCGGCTCTTC CCTTTAACTT CCAGGCTGCC  
     1501 TATGGCCTGA GTGACCAACT GGCCCAAGCC ATCAGTGACC ACTATCCAGT GGAGGTGATG  
     1561 CTGAAGGGGG GCGGACCCAA AAAGAAGCGC AAGGTTTGA

//

*Fig. 19(C)*

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```

      9      18      27      36      45      54
5' ATG GGA TGG AGC TGT ATC ATC CTC TTC TTG GTA GCA ACA GCT ACA GGT GTC CAC
---
M G W S C I I L F L V A T A T G V H

      63      72      81      90      99      108
TCC CAG GTG CAG CTG GTG CAG TCT GGG GCA GAG GTG AAA AAG CCT GGG GCC TCA
---
S Q V Q L V Q S G A E V K K P G A S

      117      126      135      144      153      162
GTG AAG GTG TCC TGC AAG GCT TCT GGC TAC ACC TTC AGT GCC TAC TGG ATA GAG
---
V K V S C K A S G Y T F S A Y W I E

      171      180      189      198      207      216
TGG GTG CGC CAG GCT CCA GGA AAG GGC CTC GAG TGG GTC GGA GAG ATT TTA CCT
---
W V R Q A P G K G L E W V G E I L P

      225      234      243      252      261      270
GGA AGT AAT AAT TCT AGA TAC AAT GAG AAG TTC AAG GGC CGA GTG ACA GTC ACT
---
G S N N S R Y N E K F K G R V T V T

      279      288      297      306      315      324
AGA GAC ACA TCC ACA AAC ACA GCC TAC ATG GAG CTC AGC AGC CTG AGG TCT GAG
---
R D T S T N T A Y M E L S S L R S E

      333      342      351      360      369      378
GAC ACA GCC GTC TAT TAC TGT GCA AGA TCC TAC GAC TTT GCC TGG TTT GCT TAC
---
D T A V Y Y C A R S Y D F A W F A Y

      387      396      405      414      423      432
TGG GGC CAA GGG ACT CTG GTC ACA GTC TCC TCA GCC TCC ACC AAG GGC CCA TCG
---
W G Q G T L V T V S S A S T K G P S

      441      450      459      468      477      486
GTC TTC CCC CTG GCA CCC TCC TCC AAG AGC ACC TCT GGG GGC ACA GCG GCC CTG
---
V F P L A P S S K S T S G G T A A L

      495      504      513      522      531      540
GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG AAC TCA
---
G C L V K D Y F P E P V T V S W N S

      549      558      567      576      585      594
GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA
---
G A L T S G V H T F P A V L Q S S G

```

**Fig. 19(D)**  
**(Sheet 1 of 3)**

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CTC	TAC	TCC	CTC	AGC	AGC	GTG	GTG	ACC	GTG	CCC	TCC	AGC	AGC	TTG	GGC	ACC	CAG
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
L	Y	S	L	S	S	V	V	T	V	P	S	S	S	L	G	T	Q
ACC	TAC	ATC	TGC	AAC	GTG	AAT	CAC	AAG	CCC	AGC	AAC	ACC	AAG	GTG	GAC	AAG	AAA
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
T	Y	I	C	N	V	N	H	K	P	S	N	T	K	V	D	K	K
GTT	GAG	CCC	AAA	TCT	TGT	GAC	AAA	ACT	CAC	ACA	TGC	TGT	GTG	GAG	TGC	CCA	CCG
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
V	E	P	K	S	C	D	K	T	H	T	C	C	V	E	C	P	P
TGC	CCA	GCA	CCT	GAA	GGC	GGG	CTG	AAG	ATC	GCA	GCC	TTC	AAC	ATC	CAG	ACA	TTT
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
C	P	A	P	E	G	G	L	K	I	A	A	F	N	I	Q	T	F
GGG	GAG	ACC	AAG	ATG	TCC	AAT	GCC	ACC	CTC	GTC	AGC	TAC	ATT	GTG	CAG	ATC	CTG
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
G	E	T	K	M	S	N	A	T	L	V	S	Y	I	V	Q	I	L
AGC	CGC	TAC	GAC	ATC	GCC	CTG	GTC	CAG	GAG	GTC	AGA	GAC	AGC	CAC	CTG	ACT	GCC
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
S	R	Y	D	I	A	L	V	Q	E	V	R	D	S	H	L	T	A
GTG	GGG	AAG	CTG	CTG	GAC	AAC	CTC	AAT	CAG	GAC	GCA	CCA	GAC	ACC	TAT	CAC	TAC
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
V	G	K	L	L	D	N	L	N	Q	D	A	P	D	T	Y	H	Y
GTG	GTC	AGT	GAG	CCA	CTG	GGA	CGG	AAC	AGC	TAT	AAG	GAG	CGC	TAC	CTG	TTC	GTG
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
V	V	S	E	P	L	G	R	N	S	Y	K	E	R	Y	L	F	V
TAC	AGG	CCT	GAC	CAG	GTG	TCT	GCG	GTG	GAC	AGC	TAC	TAC	TAC	GAT	GAT	GGC	TGC
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Y	R	P	D	Q	V	S	A	V	D	S	Y	Y	Y	D	D	G	C
GAG	CCC	TGC	GGG	AAC	GAC	ACC	TTC	AAC	CGA	GAG	CCA	GCC	ATT	GTC	AGG	TTC	TTC
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
E	P	C	G	N	D	T	F	N	R	E	P	A	I	V	R	F	F
TCC	CGG	TTC	ACA	GAG	GTC	AGG	GAG	TTT	GCC	ATT	GTT	CCC	CTG	CAT	GCG	GCC	CCG
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
S	R	F	T	E	V	R	E	F	A	I	V	P	L	H	A	A	P
1197	1206	1215	1224	1233	1242												

**Fig. 19(D)**  
**(Sheet 2 of 3)**

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```

GGG GAC GCA GTA GCC GAG ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT GTC CAA
-----
G   D   A   V   A   E   I   D   A   L   Y   D   V   Y   L   D   V   Q

      1251      1260      1269      1278      1287      1296
GAG AAA TGG GGC TTG GAG GAC GTC ATG TTG ATG GGC GAC TTC AAT GCG GGC TGC
-----
E   K   W   G   L   E   D   V   M   L   M   G   D   F   N   A   G   C

      1305      1314      1323      1332      1341      1350
AGC TAT GTG AGA CCC TCC CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC CCC ACC
-----
S   Y   V   R   P   S   Q   W   S   S   I   R   L   W   T   S   P   T

      1359      1368      1377      1386      1395      1404
TTC CAG TGG CTG ATC CCC GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG CAC TGT
-----
F   Q   W   L   I   P   D   S   A   D   T   T   A   T   P   T   H   C

      1413      1422      1431      1440      1449      1458
GCC TAT GAC AGG ATC GTG GTT GCA GGG ATG CTG CTC CGA GGG GCC GTT GTT CCC
-----
A   Y   D   R   I   V   V   A   G   M   L   L   R   G   A   V   V   P

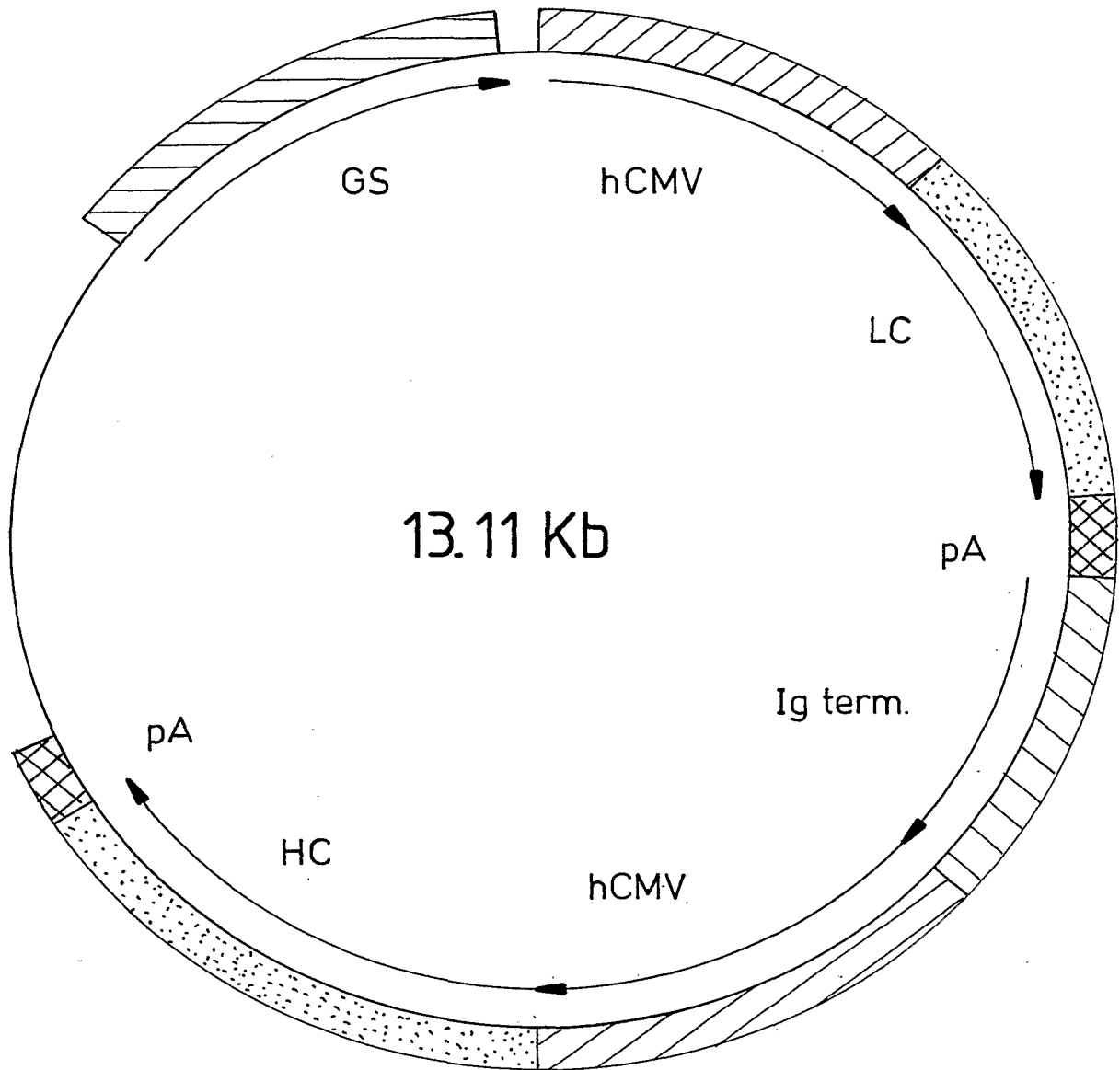
      1467      1476      1485      1494      1503      1512
GAC TCG GCT CTT CCC TTT AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC CAA CTG
-----
D   S   A   L   P   F   N   F   Q   A   A   Y   G   L   S   D   Q   L

      1521      1530      1539      1548      1557      1566
GCC CAA GCC ATC AGT GAC CAC TAT CCA GTG GAG GTG ATG CTG AAG GGG GGC GGA
-----
A   Q   A   I   S   D   H   Y   P   V   E   V   M   L   K   G   G   G

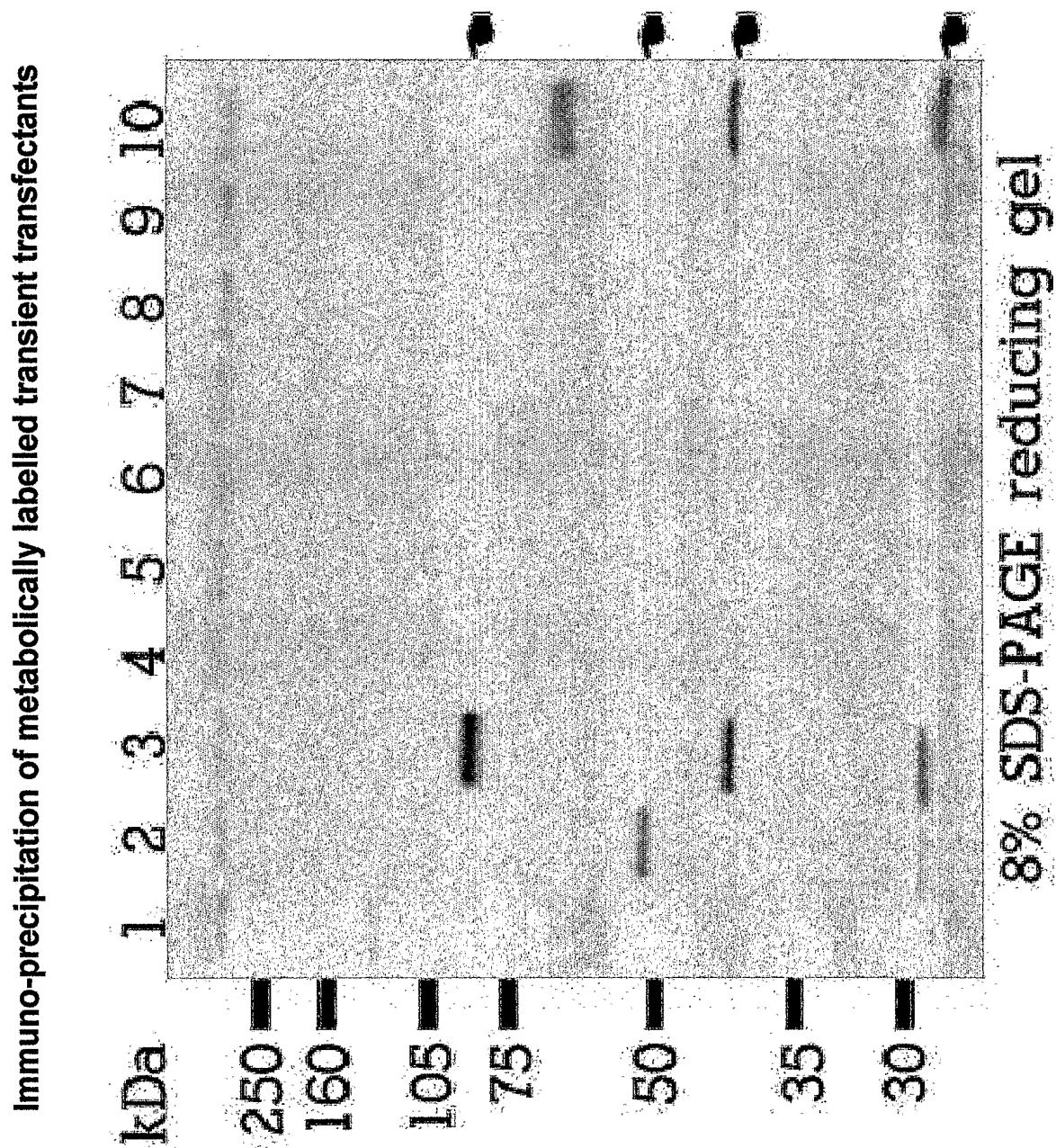
      1575      1584
CCC AAA AAG AAG CGC AAG GTT TGA 3'
-----
P   K   K   K   R   K   V   *

```

***Fig. 19(D)***  
***(Sheet 3 of 3)***

**93/113****Mammalian expression of humanised HMFG1-D Nase constructs*****Fig. 20***

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*Fig. 21(A)*

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Immuno-precipitation of metabolically labelled transient transfectants

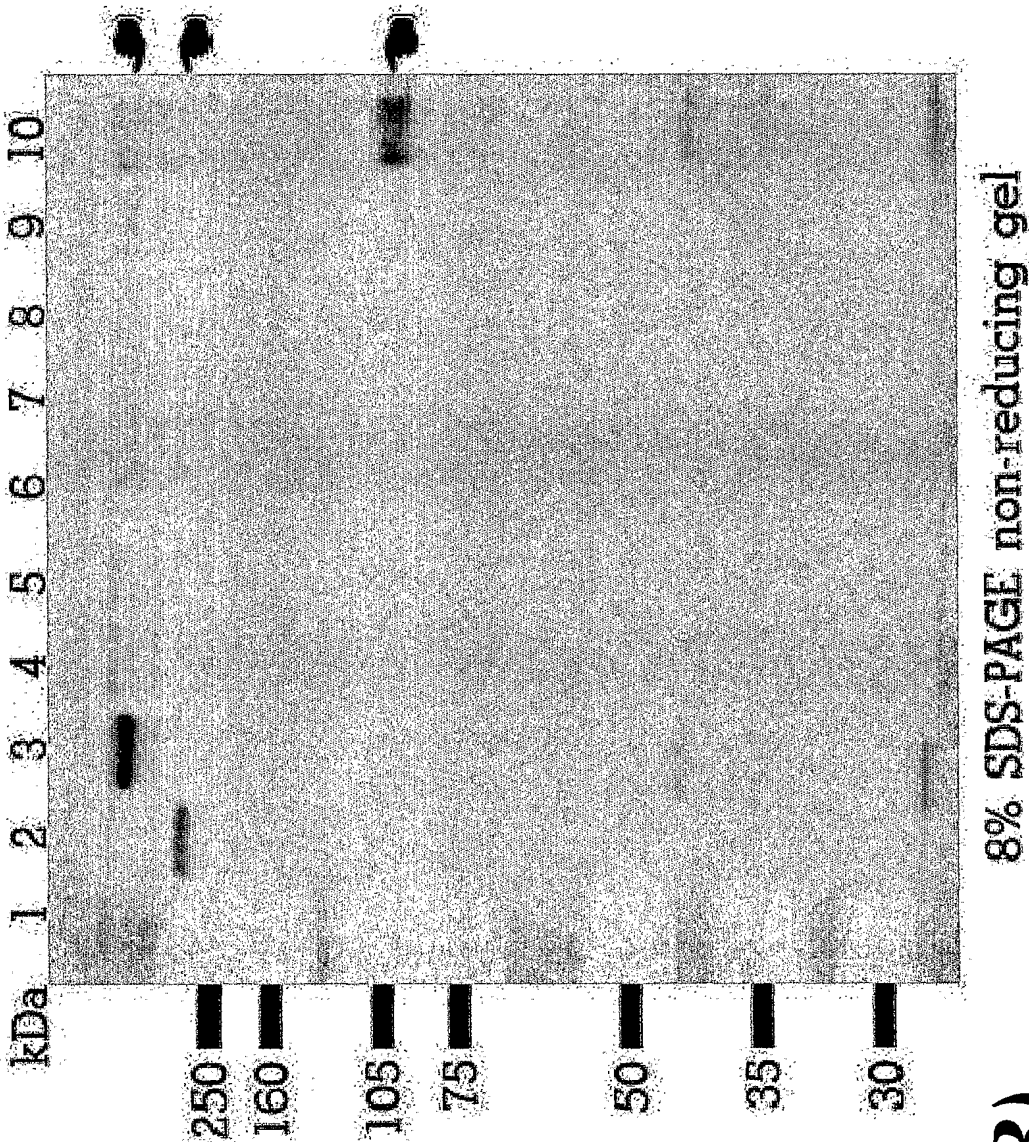


Fig. 21(B)

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Immuno-precipitation of metabolically labelled transient transfectants

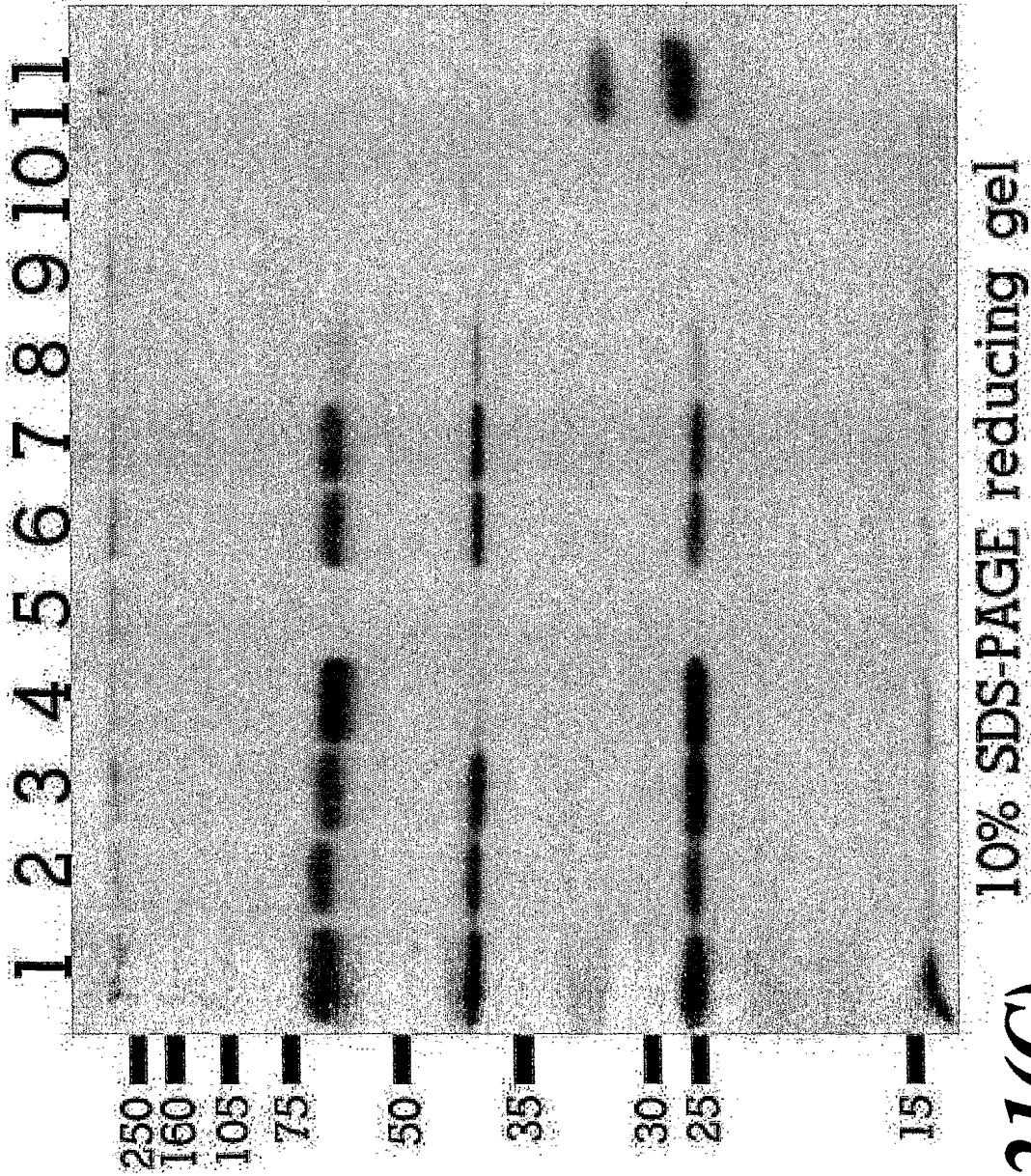
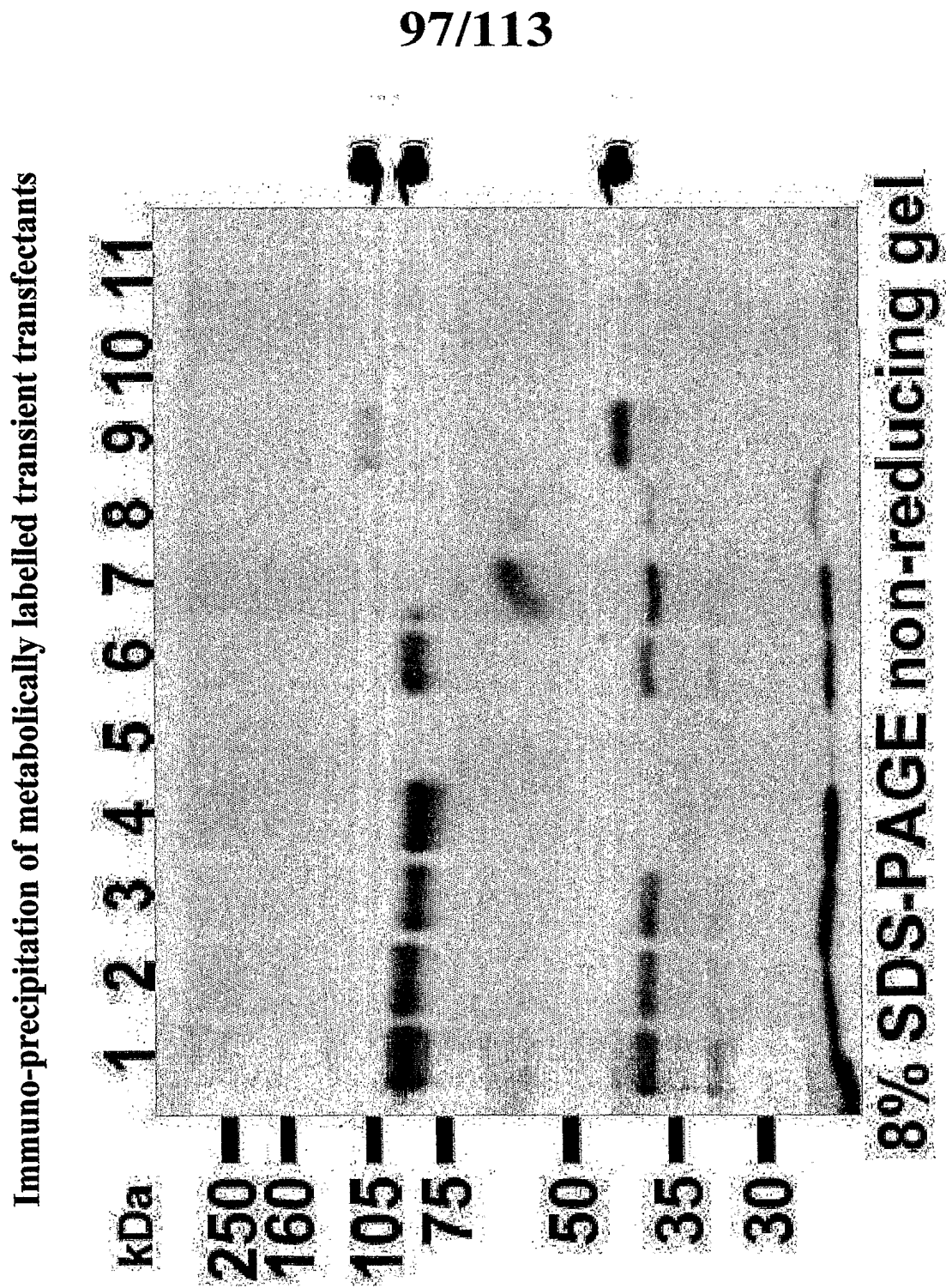
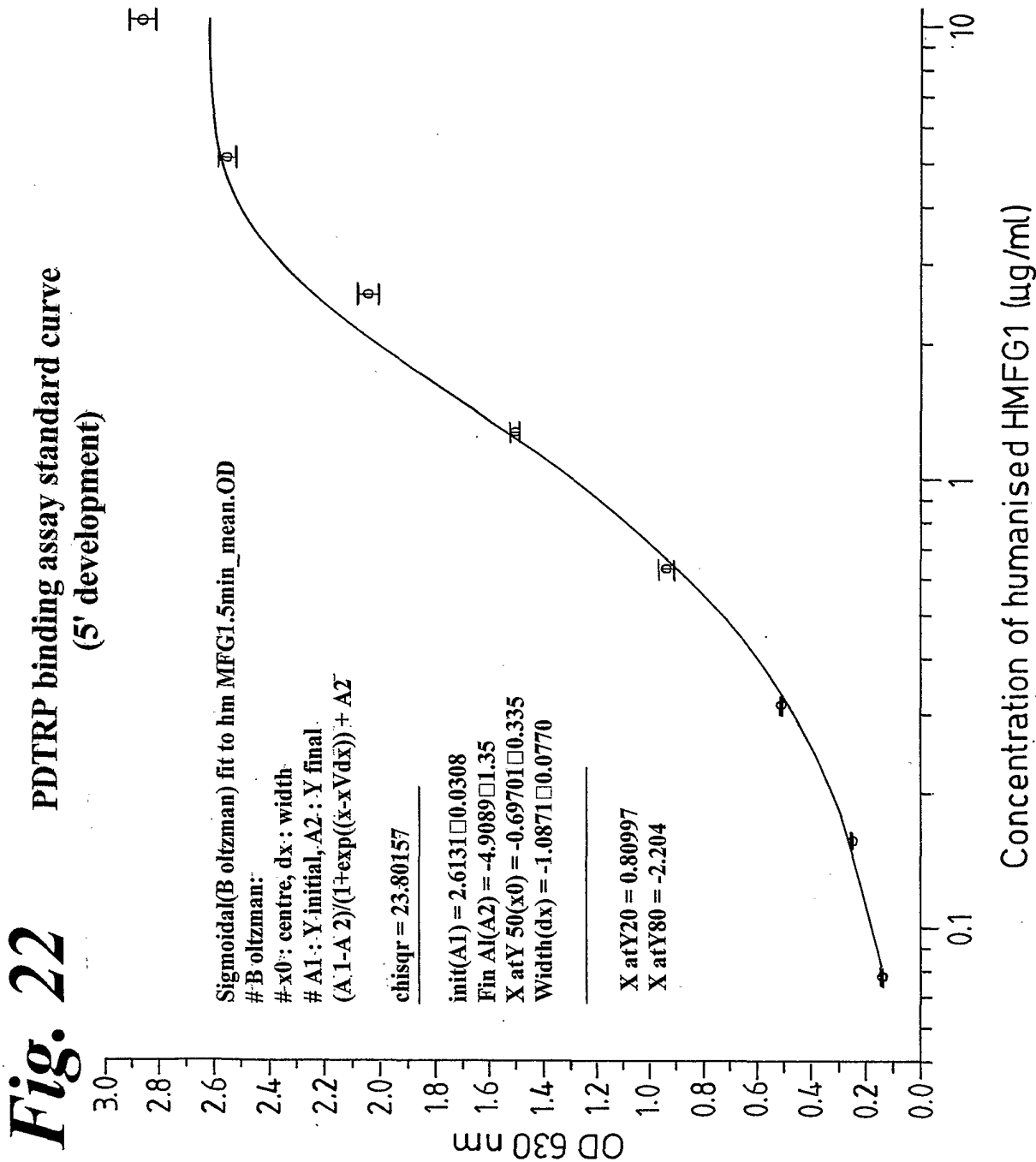


Fig. 21(C)



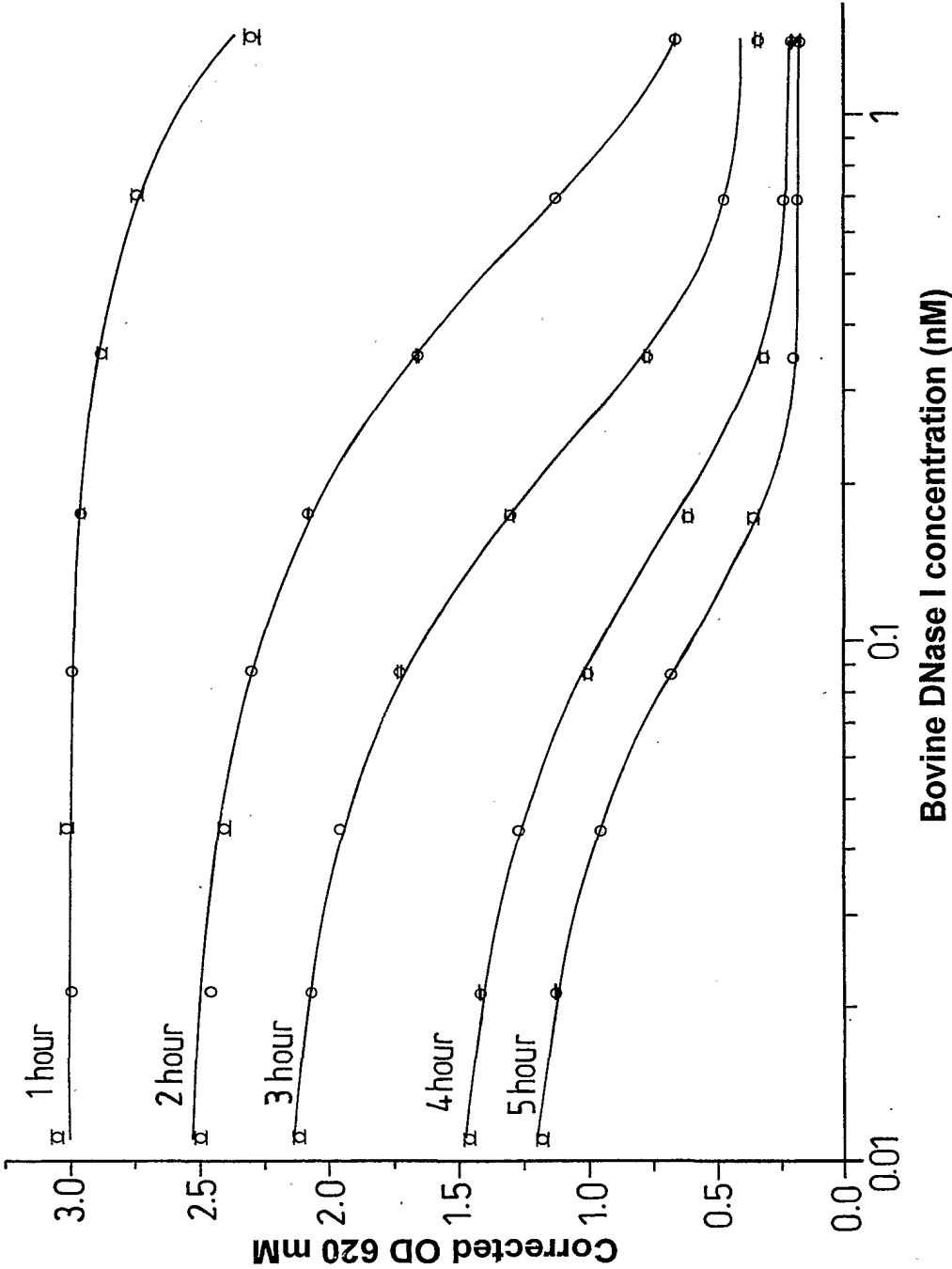
*Fig. 21(D)*

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**Fig. 23**  
Corrected bovine DNase I standard curves  
at various time points



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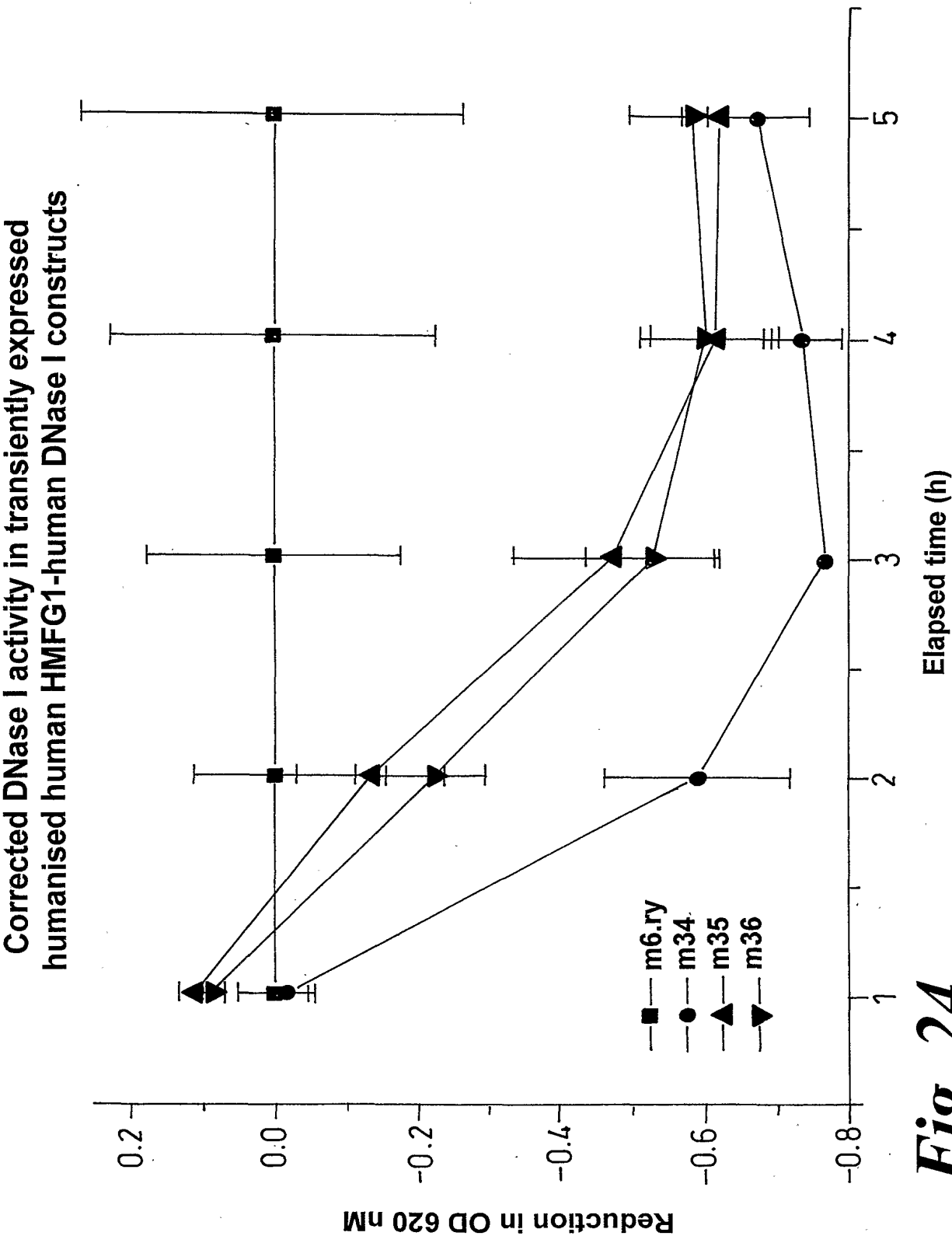
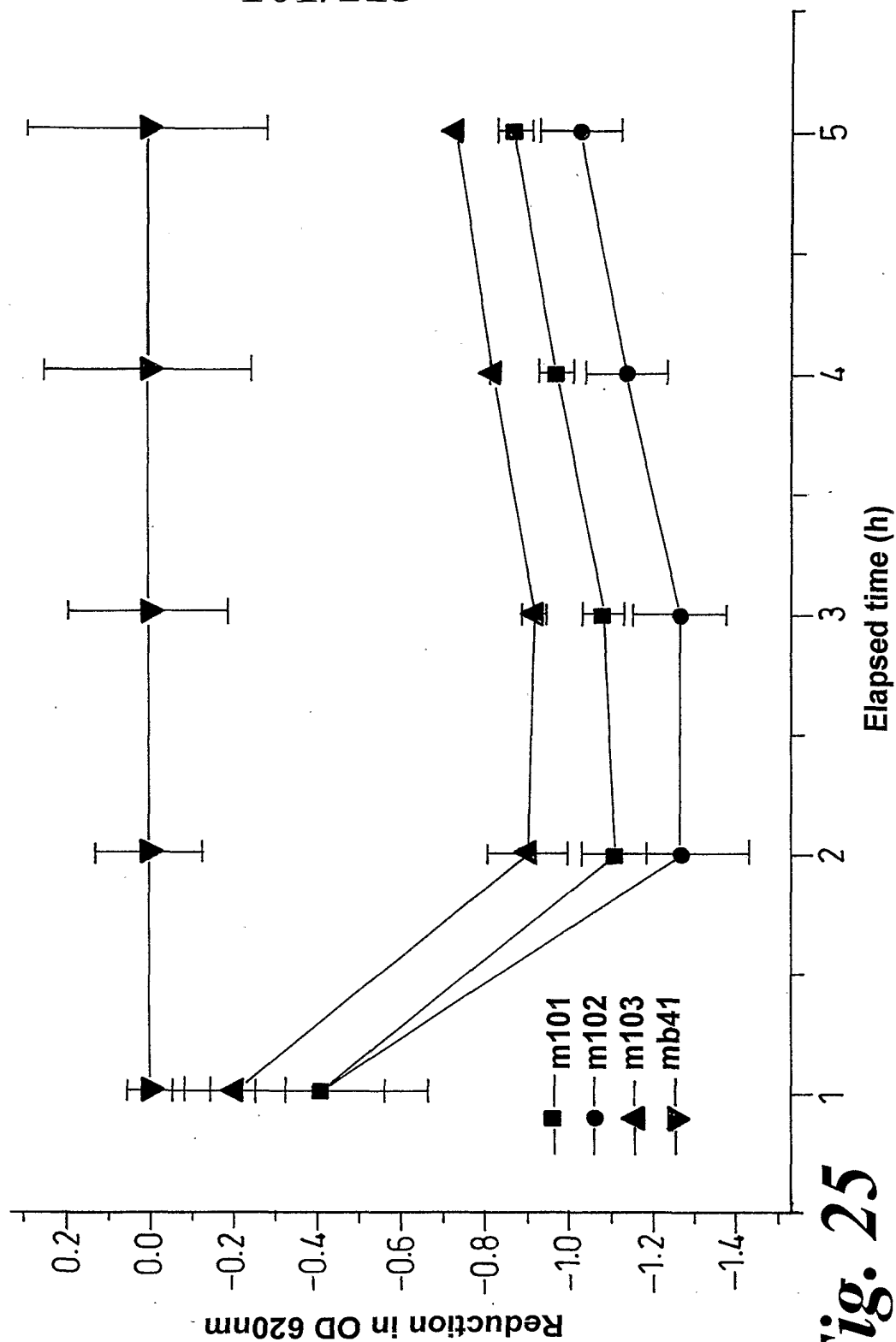


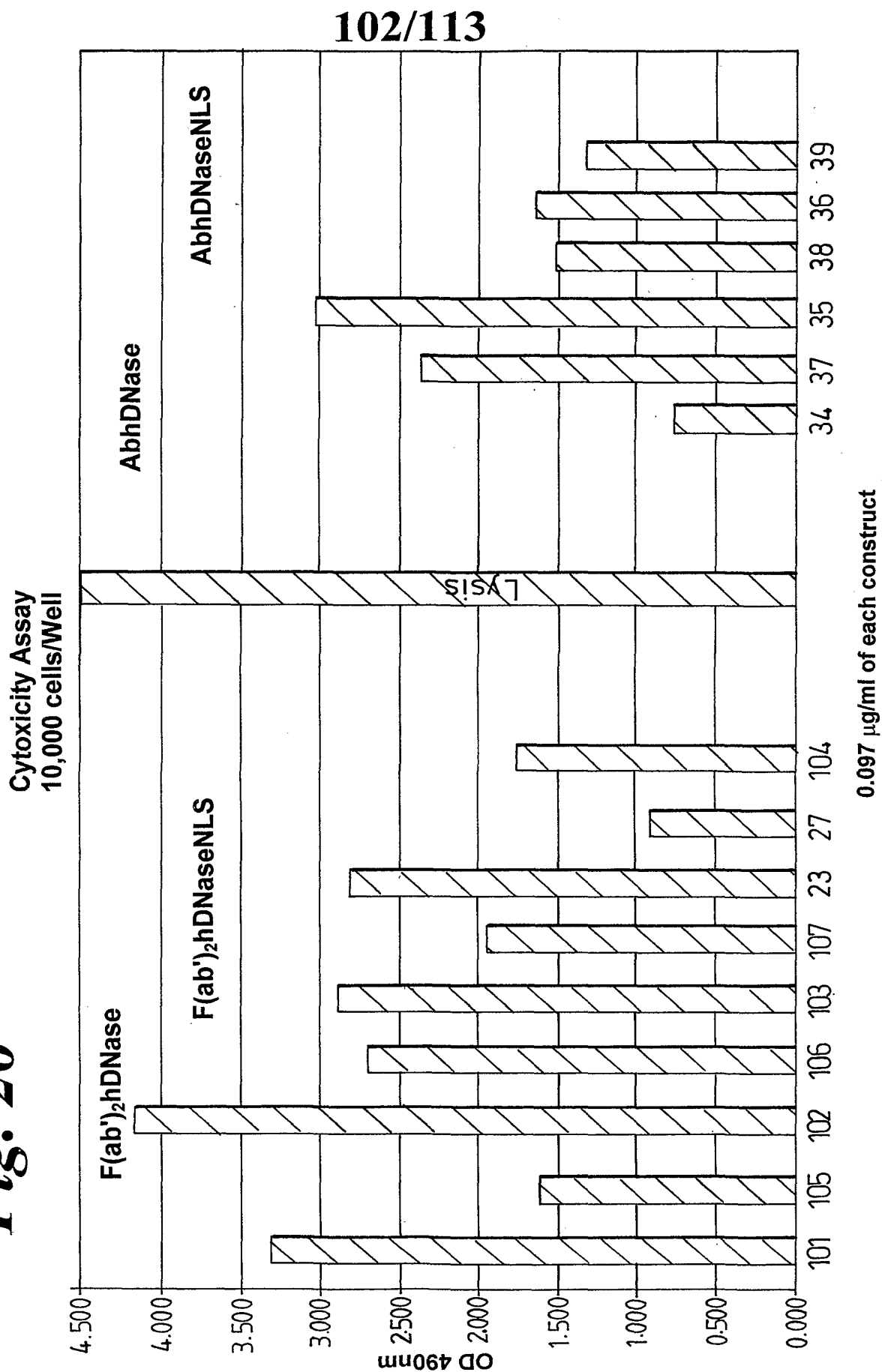
Fig. 24

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Corrected DNase I activity in transiently expressed  
humanised HMFG1 F(ab')<sub>2</sub>-human DNase I fusions

**Fig. 25**

**Fig. 26**



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MCF7 cells killed after 1h incubation with 1.35 ng of sample

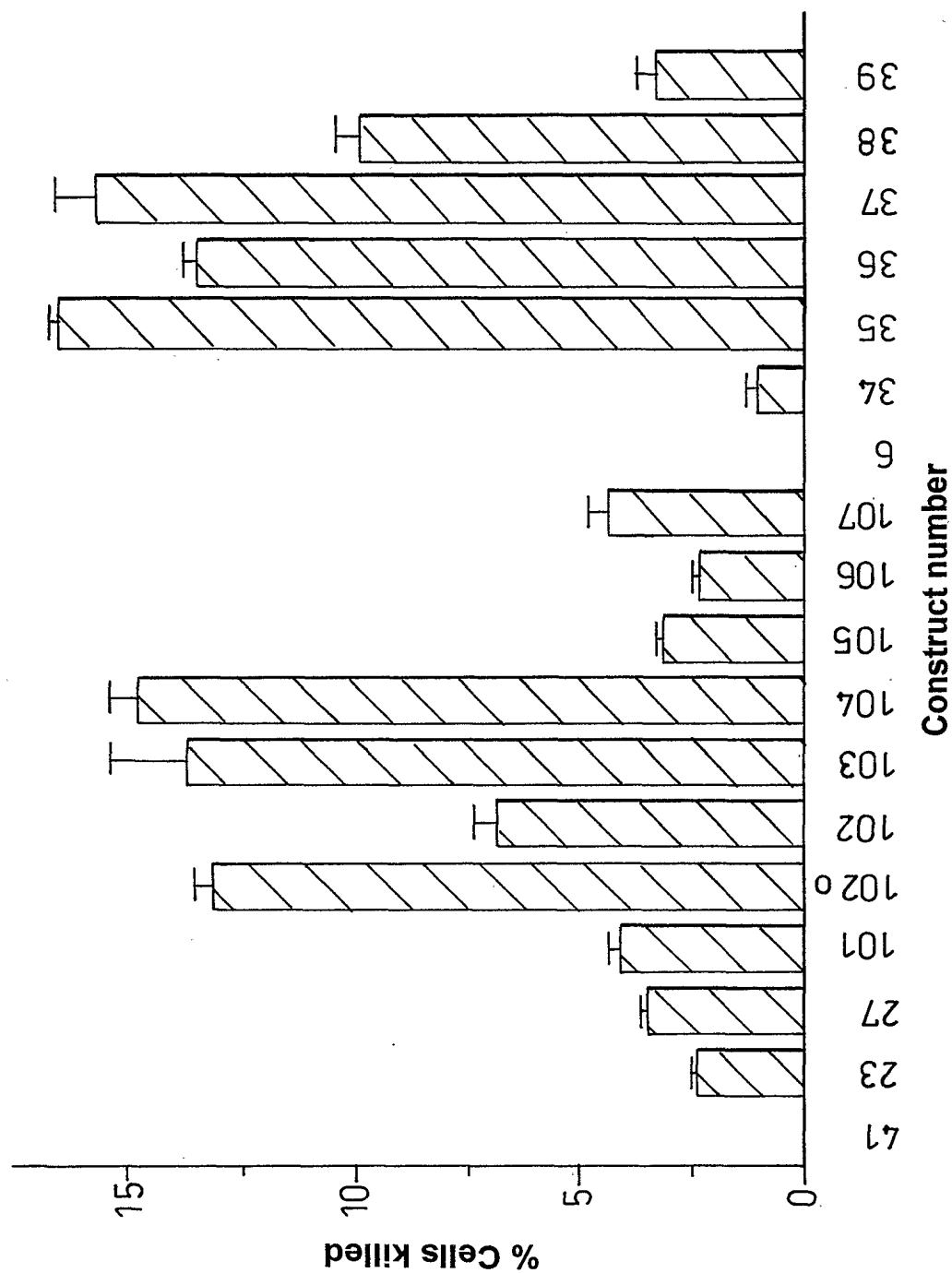
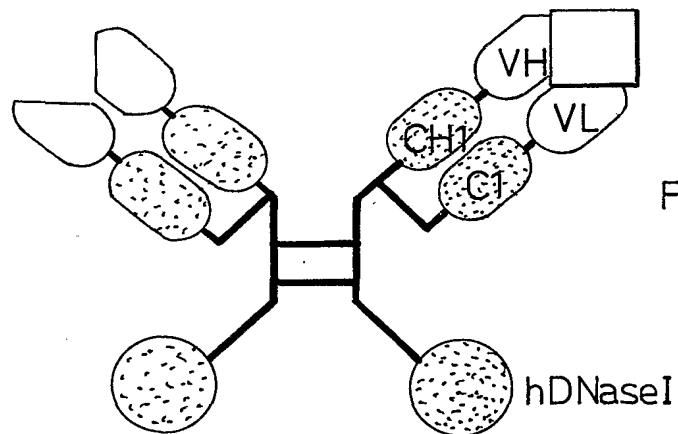
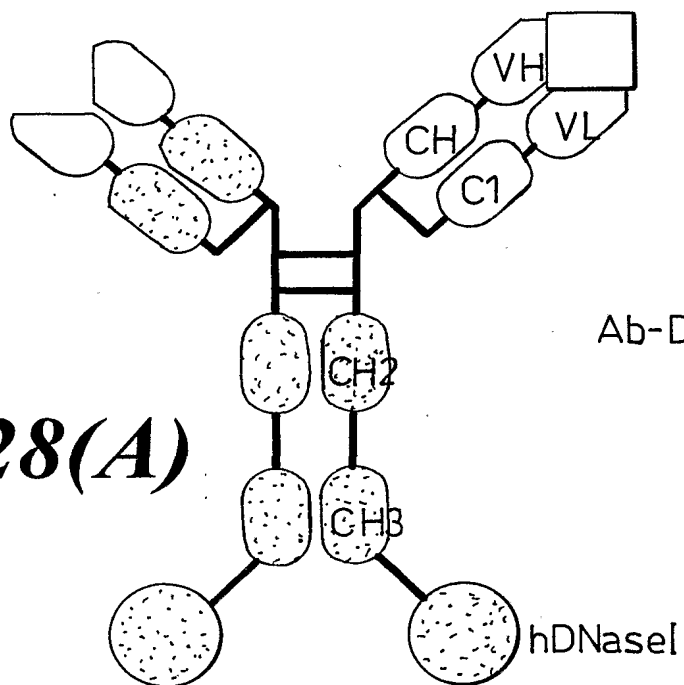
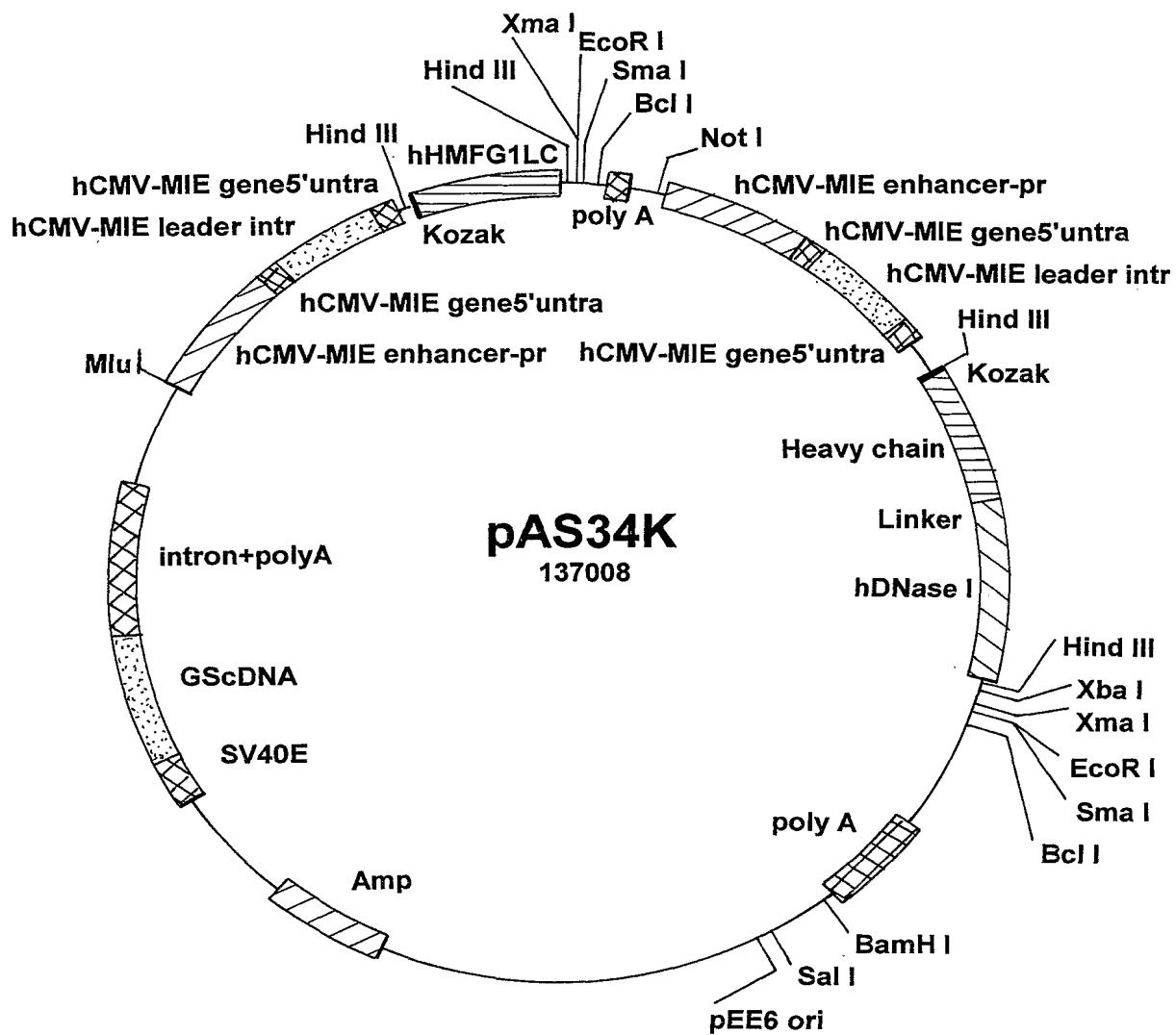


Fig. 27

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**Fig. 28(A)****Fig. 28(B)**

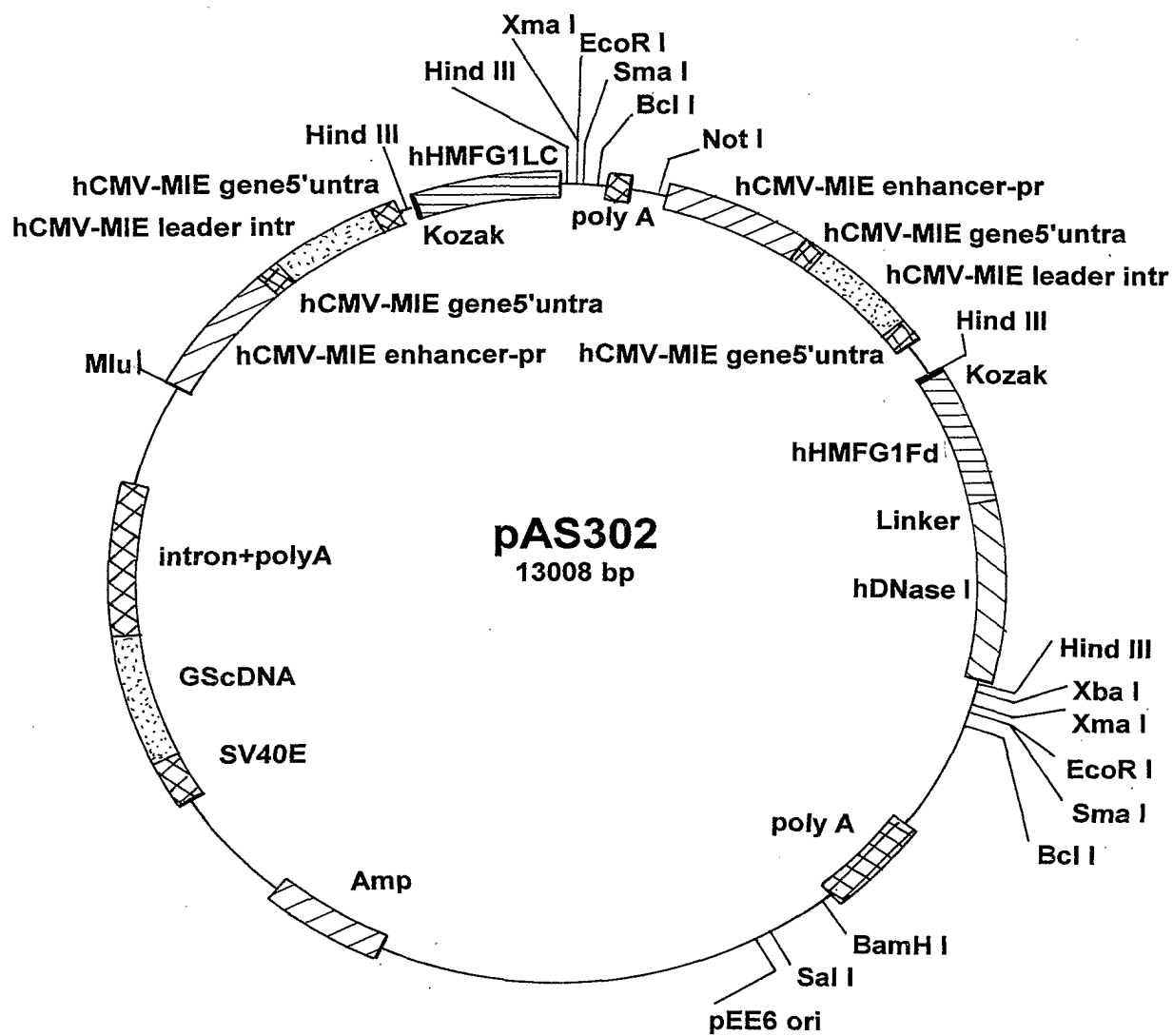
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Ab-DNase

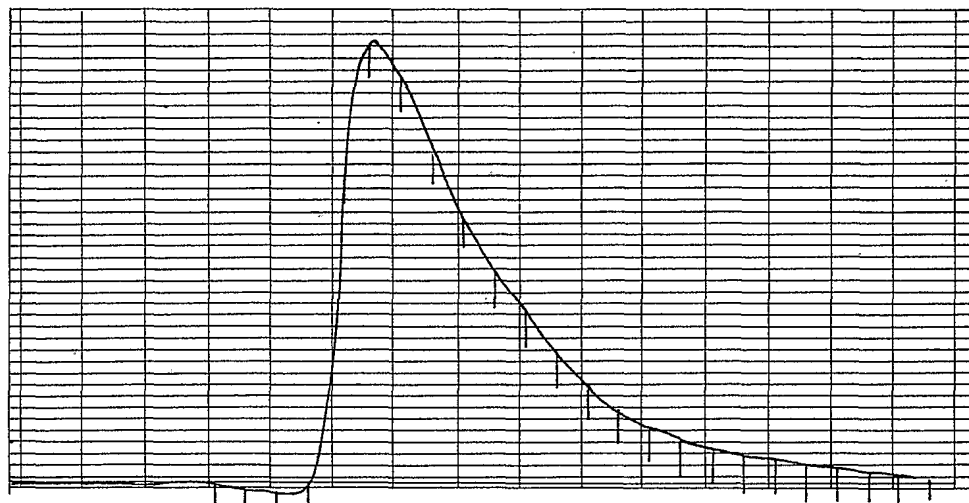
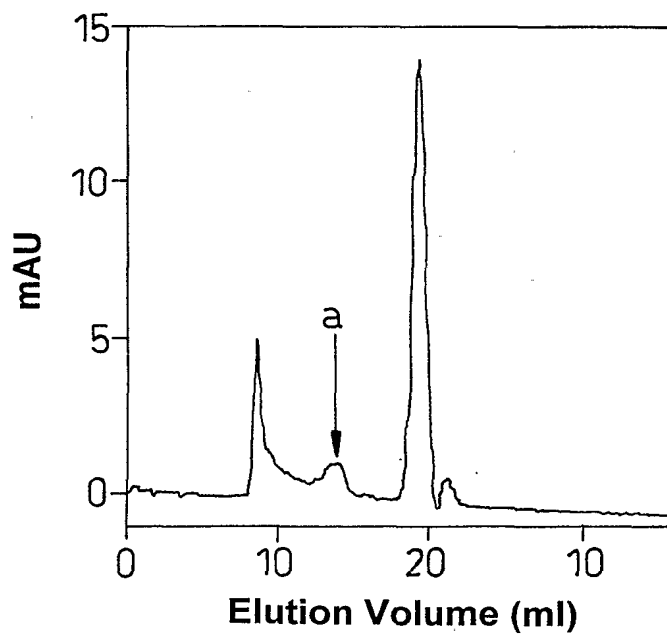
*Fig. 29*

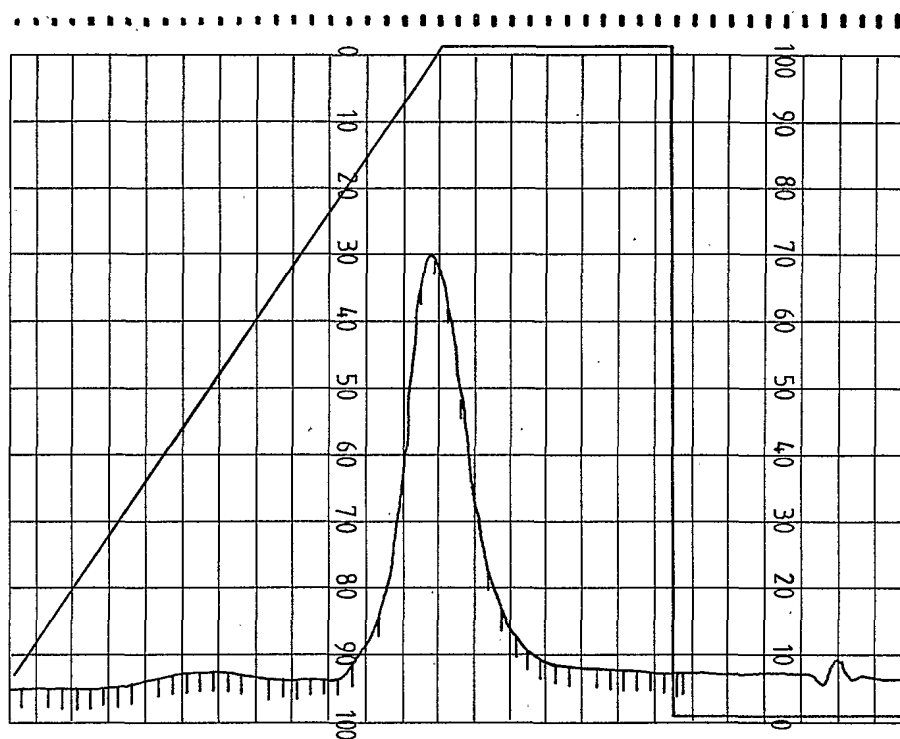
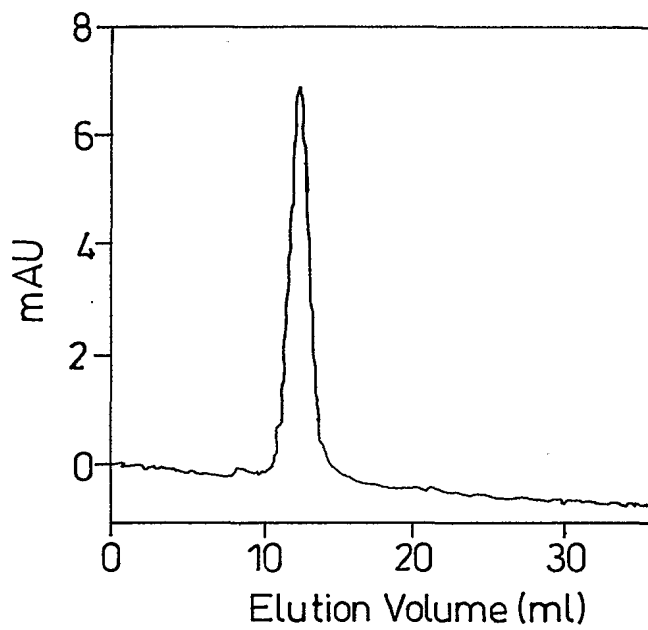
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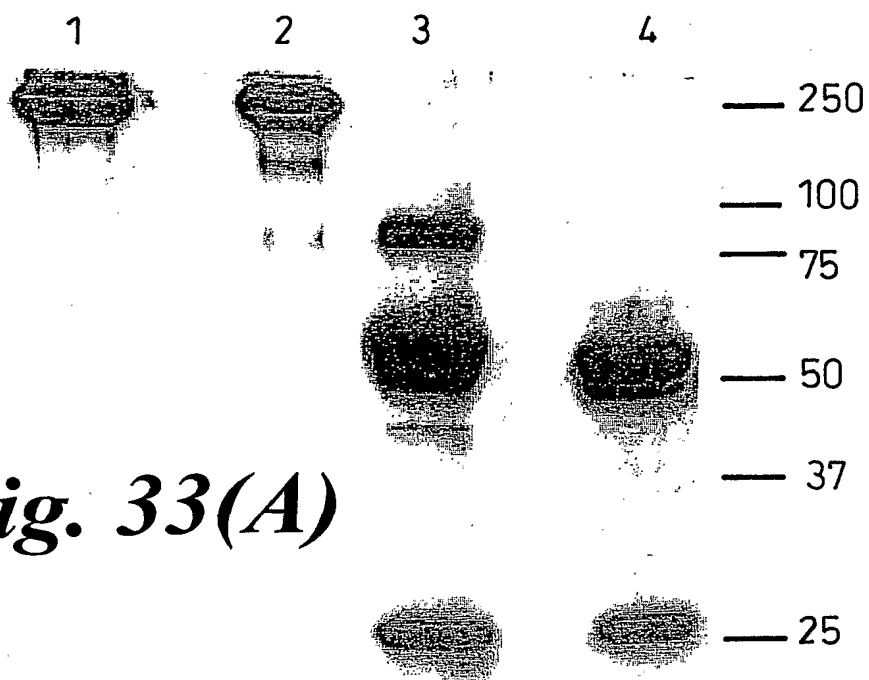
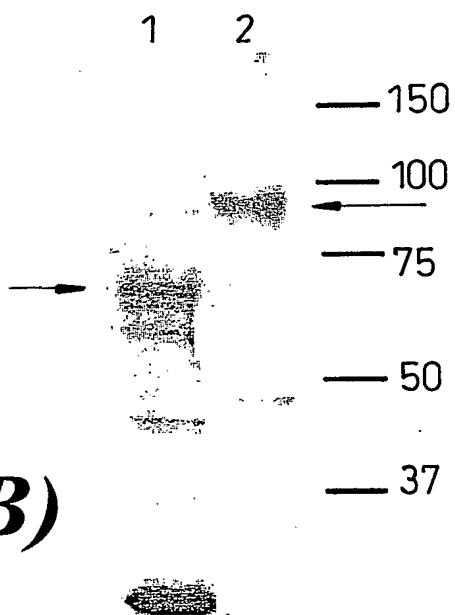


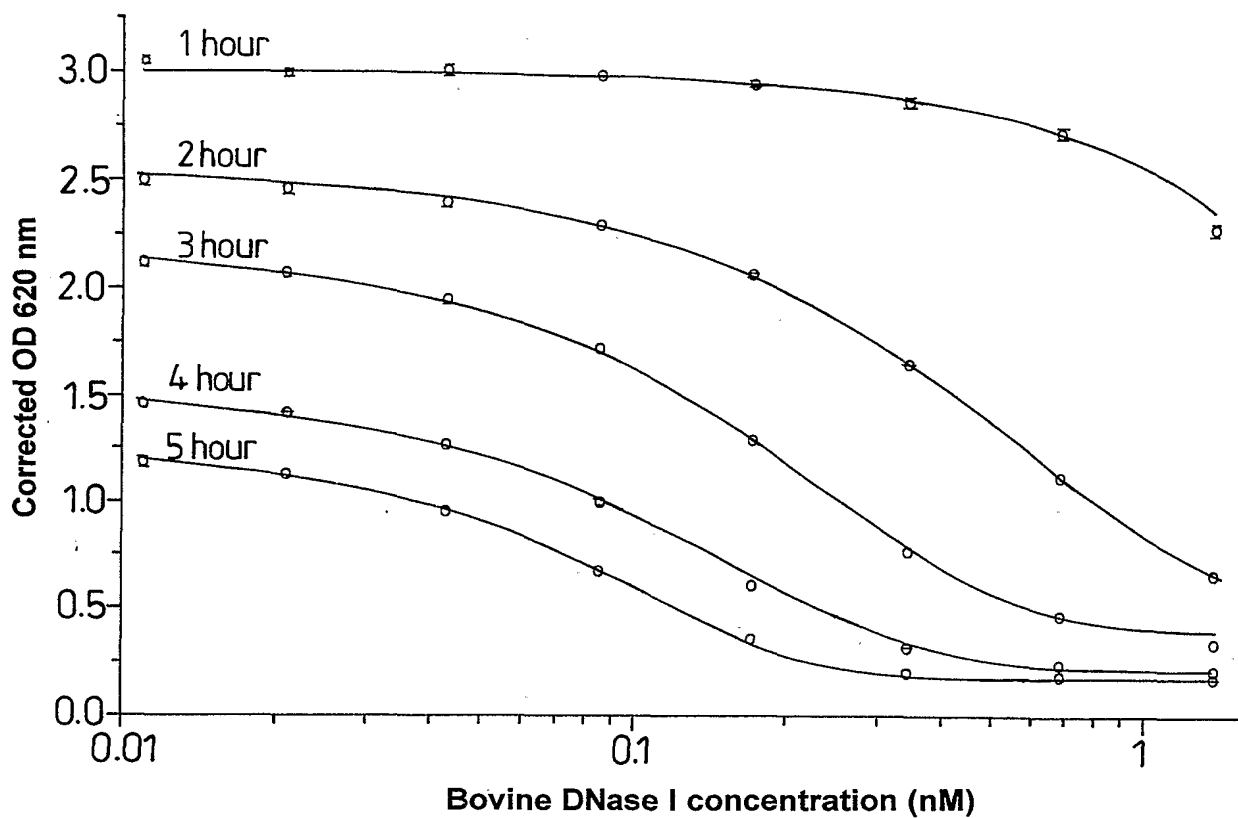
Fab-DNase

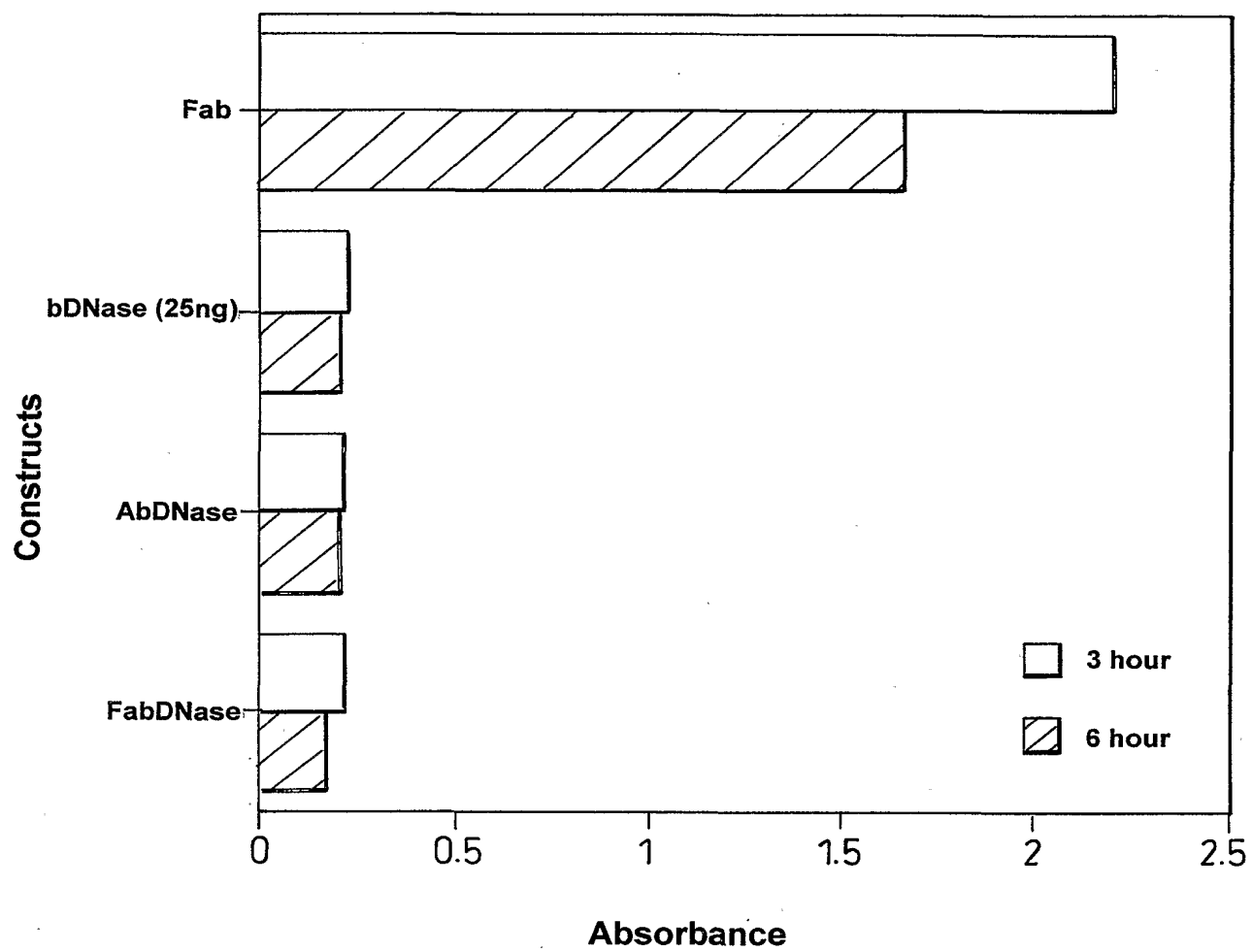
*Fig. 30*

**107/113*****Fig. 31(A)******Fig. 31(B)***

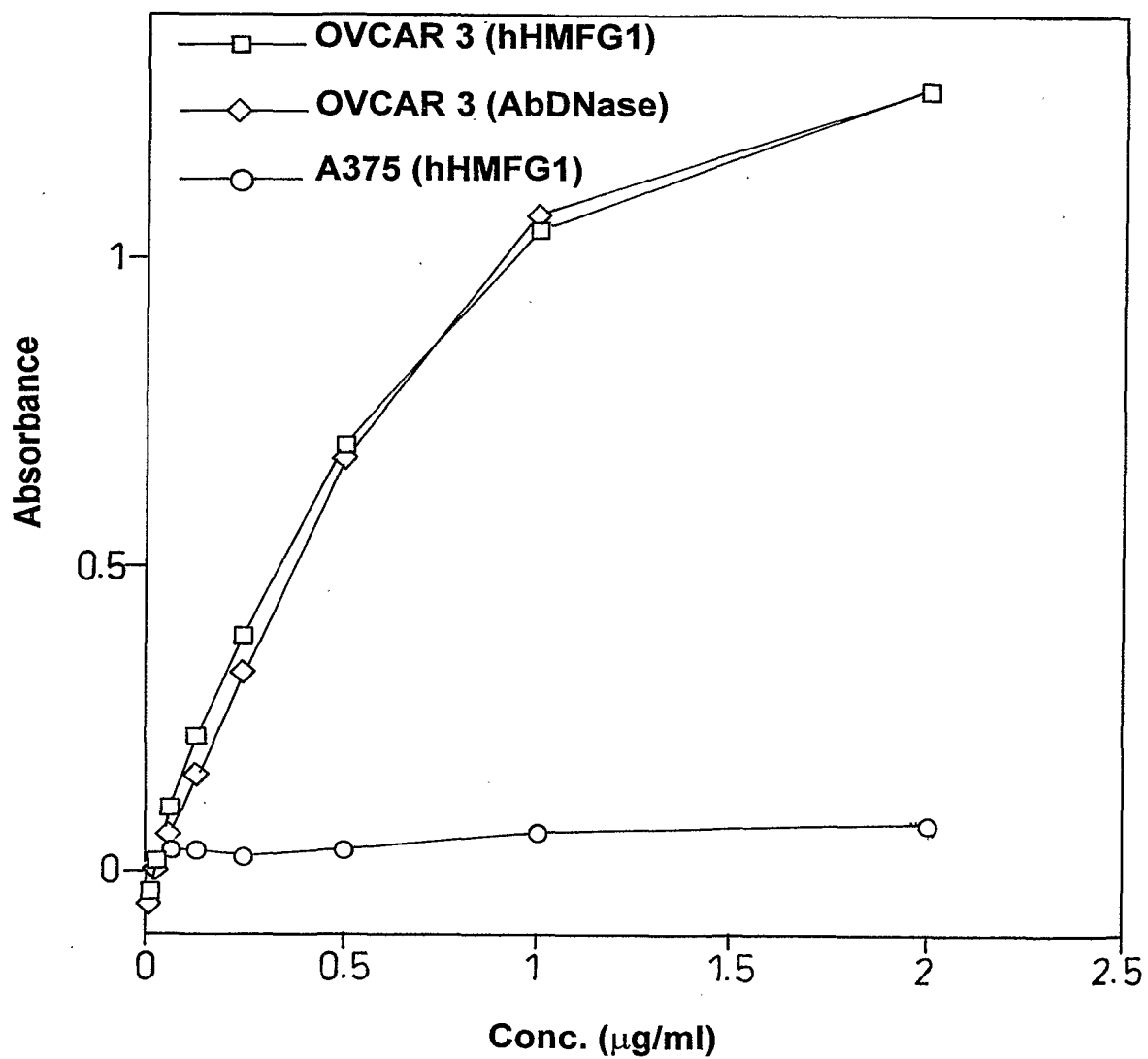
**108/113*****Fig. 32(A)******Fig. 32(B)***

**109/113*****Fig. 33(A)******Fig. 33(B)***

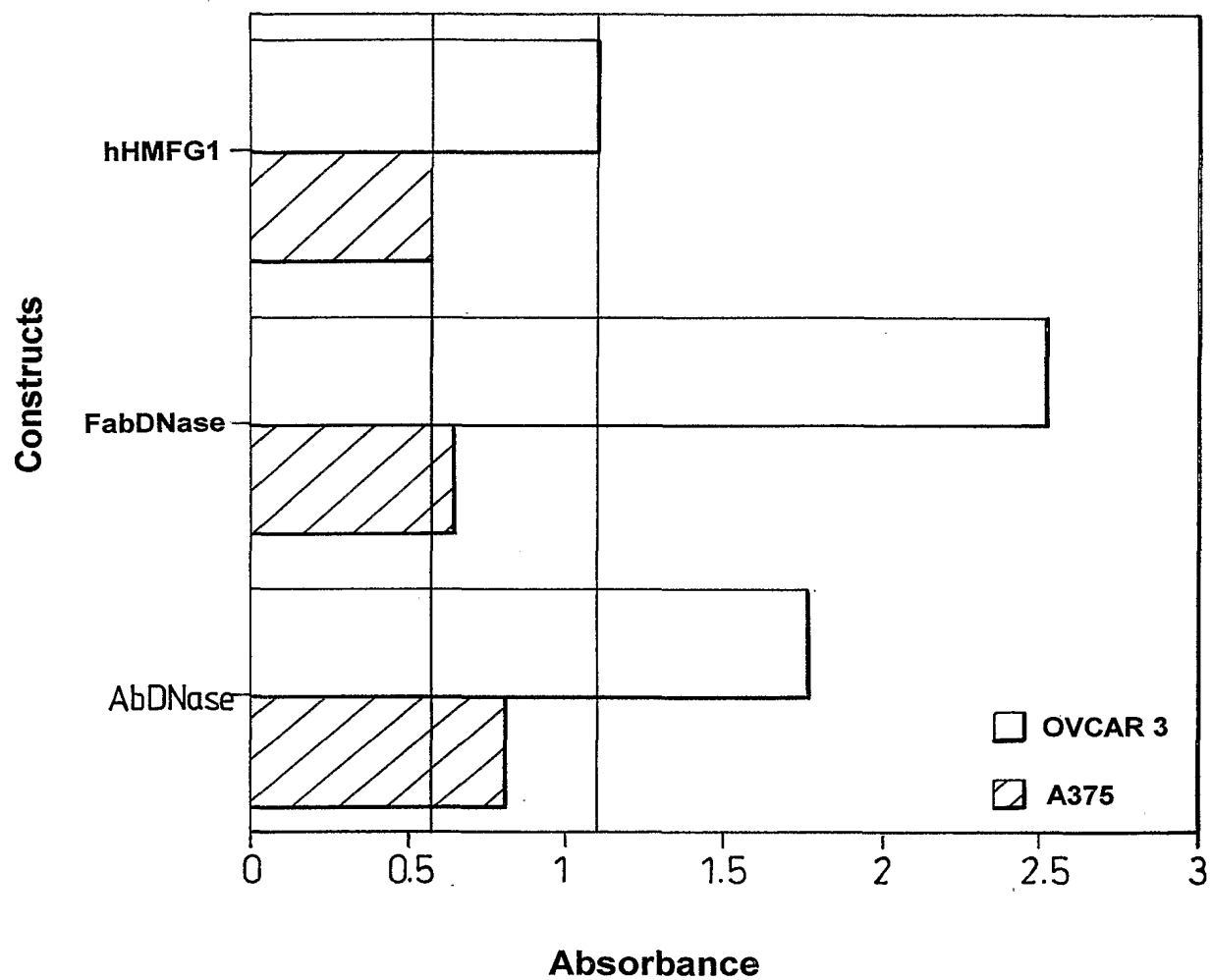
**110/113****Bovine DNase I standard curves at various time points*****Fig. 34(A)***

**111/113*****Fig. 34(B)***

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*Fig. 35(A)*

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*Fig. 35(B)*

## INTERNATIONAL SEARCH REPORT

ational Application No

PCT/GB 01/01324

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> IPC 7 C07K16/18 C12N9/22 C12N15/62 C07K16/46 C12N15/63 C12N15/85 A61K39/395 A61K38/43 //C07K19/00		
According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b> Minimum documentation searched (classification system followed by classification symbols) IPC 7 C07K C12N		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used) BIOSIS, EPO-Internal, WPI Data, MEDLINE, PAJ		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	YOUNG ROBERT J ET AL: "A DNase I based immunotoxin for tumor therapy." PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH ANNUAL, no. 41, March 2000 (2000-03), page 289 XP001008862 91st Annual Meeting of the American Association for Cancer Research.; San Francisco, California, USA; April 01-05, 2000, March, 2000 ISSN: 0197-016X abstract  --- -/--	1-12, 14, 15, 17, 21, 23, 25, 28-35, 37, 38
<input checked="" type="checkbox"/> Further documents are listed in the continuation of box C.		
<input checked="" type="checkbox"/> Patent family members are listed in annex.		
° Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "&" document member of the same patent family		
Date of the actual completion of the international search		Date of mailing of the international search report
6 August 2001		16/08/2001
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016		Authorized officer  Montrone, M

## INTERNATIONAL SEARCH REPORT

ational Application No

PCT/GB 01/01324

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>WO 94 15644 A (EPENETOS AGAMEMNON ANTONIOU ;IMP CANCER RES TECH (GB); DEONARAIN M)  21 July 1994 (1994-07-21)  abstract  page 3, line 12-22  page 4, line 24-26  page 6, line 7 -page 8, line 2  page 10, line 11-14  page 12, line 18 -page 13, line 8  page 15, line 1-19  page 26, line 30 -page 27, line 25  page 28, line 23 -page 29, line 21  page 49, line 29 -page 51, line 10</p>	<p>1-6,  9-19, 23,  28-38</p>
Y	<p>LINARDOU H. ET AL.: "Deoxyribonuclease I (DNase I). A novel approach for targeted cancer therapy."  CELL BIOPHYS.,  vol. 24-25, 1994, page 243-248 XP001012902  abstract  page 244, paragraphs 2-4  page 245, paragraph 4  page 246; figure 1  page 247, paragraphs 2,3,5</p>	<p>15,16,  18,19</p>
Y	<p>WO 92 04380 A (UNILEVER PLC ;UNILEVER NV (NL)) 19 March 1992 (1992-03-19)  cited in the application  abstract  page 4, line 30 - line 27  page 6, line 6-26  page 8, line 30 -page 9, line 7  page 9, line 11-28  page 10, line 1-6  page 10, line 33 -page 11, line 3  page 12, line 13-35  page 14, line 14-16  page 15, line 15-30  page 16, line 15-20  page 17, line 6-14</p>	<p>1-19, 21,  23, 25,  27-38</p>
Y	<p>EP. 0 781 845 A (CELLTECH THERAPEUTICS LTD)  2 July 1997 (1997-07-02)  abstract  page 3, line 19-48  page 5, line 3 -page 6, line 15  page 9, line 23-42  page 16, line 8-17</p>	<p>1-19, 21,  23, 25,  27-38</p>

**FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210**

Continuation of Box I.2

Claims Nos.: 20,22,24,26,38

Present claims 20, 24 and 38 relate to an extremely large number of possible compounds. In fact, the claims contain so many options, variables, possible permutations and provisos that a lack of clarity within the meaning of Article 6 PCT arises to such an extent as to render a meaningful search of the claims impossible. Moreover, a search for the subject-matter of claims 22 and 26 has not been carried out since it was not possible to identify the corresponding SEQ.ID.NO. of fig. 14(c). Consequently, the search has been carried out for those parts of the application which do appear to be clear, namely 1 to 19, 21, 23, 25, 27 to 37.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

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